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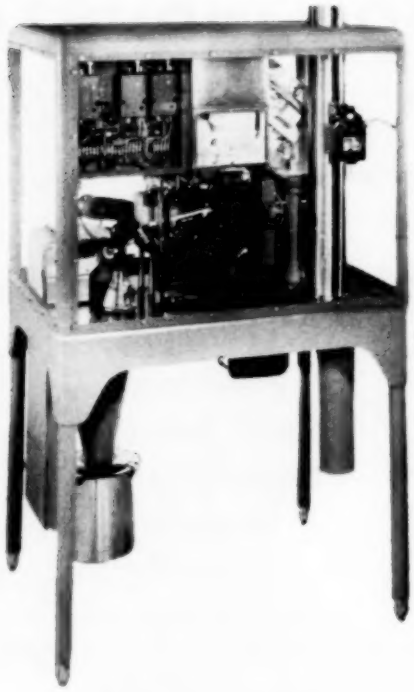

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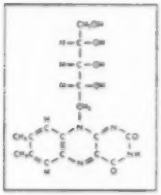
INSIDE SCIENCE

The Vital Story

A Quick History. Independent investigators, working separately to unlock several of nature's doors, sometimes open up unsuspected relationships. This happened with vitamin B_2.

Investigations. About 25 years ago, several groups, notably Warburg's, were investigating a "yellow enzyme" obtained from yeast. Almost simultaneously other investigators were studying a food factor that aided growth of laboratory animals.

What they found. Proceeding with chemical analysis of this growth factor, the team of Kuhn, György, and Wagner-Jauregg noted a relationship between the growth-producing agent and the "yellow enzyme." Their findings, and those of other researchers along similar lines, were published in 1933. Eventually, riboflavin and an essential part of the yellow enzyme were found to be identical and the unity of an essential nutrient and cellular metabolism was established.



Isolation of pure riboflavin was achieved by Kuhn and his co-workers, and by Ellinger and Koschara, in 1933.

Nomenclature. Known in the United States as riboflavin, this vitamin has also been called lactoflavin, ovoflavin, hepatoflavin, and vitamin G.

SYNTHESIS

By 1935, two eminent chemists, working separately, had synthesized riboflavin, practically in a dead heat. Prof. Paul Karrer of the University of Zurich, a collaborator of the Hoffmann-La Roche Laboratories, produced the first successful synthesis. Five weeks later Richard Kuhn of Germany announced his synthesis of the vitamin. Prof. Karrer subsequently shared the Nobel Prize in Chemistry for his work in vitamins and carotenoids.

The Karrer synthesis forms the basis for chemical processes in widespread use today by Hoffmann-La Roche and other leading manufacturers throughout the world. Riboflavin is also manufactured today by fermentation methods.

CHEMICAL AND PHYSICAL PROPERTIES

Riboflavin is yellow, slightly water-soluble with a greenish fluorescence and a bitter taste. Its empirical formula is $C_{17}H_{20}N_4O_6$. Vitamin B_2 produced by the Roche process is identical in every way with that occurring in nature.

How does vitamin B_2 work? Riboflavin is a vital part of nature's chain of reactions for utilization of carbohydrate energy. It has been found to be a constituent of many enzyme systems and is thus intimately connected with life processes. It is probably required by the metabolic processes of every animal and bird as well as by many fishes, insects and lower forms of life. (In certain animals, however, the requirement may be synthesized by bacteria within the intestine.)

In the cells riboflavin goes to work attached to a phosphate group. This substance, known as riboflavin-5′-phosphate or flavin mononucleotide, may in turn be attached to still another essential substance, adenylic acid, forming flavin adenine dinucleotide. Either nucleotide then is attached to protein, thereby forming an enzyme, and takes its part in oxidation-reduction reactions.

Requirements in Human Nutrition. As we have seen, vitamin B_2 is essential to life. We have no special storage organs in our bodies for this vitamin, although a certain level is maintained in various tissues, with relatively large amounts found in the liver and kidneys.

MEASURING METHODS

In the beginning, riboflavin activity was described in "Bourquin-Sherman units" and requirements were thought to be very small. Subsequent research showed otherwise. Milligrams of weight became the unit and the Food & Drug Administration of the U. S. Dept. of Health, Education & Welfare established (July 1, 1958) a minimum daily requirement of 1.2 mg. of riboflavin for all persons 12 or more years old. For infants it is 0.6 mg. These requirements are designed to prevent the occurrence of symptoms of riboflavin deficiency disease. The minimum daily requirement for this vitamin for children from 1 to 12 years is 0.9 milligram.

Recommended allowances. The Food & Nutrition Board of the National Research Council has recommended the following daily dietary allowances of riboflavin, expressed as milligrams. These are designed to maintain good nutrition of healthy persons in the U. S. A.

			Boys	Girls
Men			1.6	
Women			1.4	
"	(3rd trimester of pregnancy)		2.0	
"	(Lactating)		2.5	
Infants,	1-3 months		0.4	
"	4-9 "		0.7	
"	10-12 "		0.9	
Children,	1-3 years		1.0	
"	4-6 "		1.2	
"	7-9 "		1.5	
Adolescents,	10-12 years		1.8	1.8
"	13-15 "		2.1	2.0
"	16-20 "		2.5	1.9

# of VITAMIN B_2

by Science Writer

(Riboflavin)

Deficiencies of vitamin B_2 appear in several ways in human beings. The eyes, the skin, the nerves, and the blood show the effects of too little riboflavin. Laboratory animals have demonstrated that a riboflavin-deficient diet can cause death of adults and can slow or stop growth in the young. Female animals, deprived of riboflavin in the diet, may produce offspring with congenital malformations.

Medical uses. To overcome and control deficiencies in human beings, physicians have pure riboflavin available for administration by injection or orally, by itself or with other "B" vitamins or multi-vitamin-mineral combinations.

How do we get our daily riboflavin? Vitamin B_2 has wide distribution throughout the entire animal and vegetable kingdoms. Good sources are milk and its products, eggs, meats, legumes, green leaves and buds. Whole-grain cereals have significant but not large amounts of riboflavin.

ADDITION TO FOODS

Cereal foods play a large part in our diet. To produce the white flour almost all of us want, millers are obliged to remove parts of the wheat that contain much of the grain's riboflavin and other nutrients. In addition, cereal grains are not rich sources of riboflavin. Millers meet this problem by *enriching* the grain foods for which federal standards exist with vitamins B_1, B_2, niacin and the mineral iron. In the case of vitamin B_2, however, they do more than *restore* the processed food to its natural riboflavin level; they *fortify* the food with enough of this essential vitamin to make it nutritionally more valuable than it was in nature.

Acting to protect the good health of millions of Americans, bakers and millers adopted *enrichment* of white bread and white flour in 1941. Since that time, other foods, such as macaroni products, corn meal and grits, farina, pastina and breakfast cereals have had their food value increased by enrichment with pure riboflavin and other vitamins and minerals.

When enriching, fortifying or restoring, food manufacturers add the necessary quantity of riboflavin (and other vitamins and minerals) to the food during processing, so that the finished product meets federal, state, and territorial requirements or contributes to the consumer an amount of the vitamin that dietary experts believe significantly useful.

PRODUCTION

Prof. Karrer's synthesis of riboflavin was a laboratory success. Adapting the process to commercial production, however, demanded original thinking by chemists at Hoffmann-La Roche. The production of riboflavin by chemical synthesis requires the production of ribose, a rare sugar, at an early stage in the process. This special sugar must be made inexpensively if the synthesis is to be practical. Sugar chemistry is a difficult matter. In a brilliant piece of work, the Roche chemical experts developed a method to produce ribose on a commercial scale by an electrolytic process, thus overcoming a most troublesome problem. Subsequently, Roche chemists developed the first practical synthesis for riboflavin-5′-phosphate, identical with natural flavin mononucleotide.

Picture three streams joining to form a river and you have a simplified idea of the Roche process for synthesizing vitamin B_2. O-xylene and glucose are processed separately to form xylidine and ribose respectively. These are joined to form ribitylxylidine, which is then converted to ribitylaminoxylidine. Starting separately with malonic ester, which is processed through intermediate stages to alloxan, the third "stream" is then joined with ribitylaminoxylidine to form riboflavin. Purification occurs at each step of the synthesis. Riboflavin 'Roche' equals or exceeds U. S. P. standards.

By the tons. So efficient is the Roche process that pure riboflavin is produced *by the tons* for use in pharmaceutical products and processed foods. An interesting development by Roche is the production of riboflavin in different forms related to the method of end use. 'Roche' Regular riboflavin U. S. P. is especially useful in dry enrichment premixes, powdered dietary supplements, pharmaceutical tablets and soft gelatin capsules. 'Roche' Solutions type is preferred for the manufacture of solutions having low concentration. 'Roche' Riboflavin-5′-Phosphate Sodium is a highly and rapidly soluble riboflavin compound favored for all pharmaceutical liquid products and some tablets, lozenges, and capsules. It has a more pleasant taste than the bitter U. S. P. riboflavin.

This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better using pure riboflavin 'Roche.' Reprints of this and others in the series will be supplied on request without charge. Also available without cost is a brochure describing the enrichment or fortification of cereal grain products with essential vitamins and minerals. These articles and the brochure have been found most helpful as sources of accurate information in brief form. Teachers especially find them useful in education. Regardless of your occupation, feel free to write for them. Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 1956 Bourdon St., St. Laurent, P. Q.

CEREAL CHEMISTRY

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FLOUR LIPIDS AND THE BROMATE REACTION[1]

D. K. CUNNINGHAM AND I. HLYNKA

ABSTRACT

The influence of the lipids of flour upon the bromate reaction in dough has been shown to be threefold: prolonged mixing in the presence of lipids accelerates the subsequent bromate reaction; in the absence of lipid the bromate reaction is depressed; bromate reaction in doughs from defatted flours mixed in air or oxygen is depressed, presumably through concurrent removal of natural antioxidants in the lipid fraction.

The lipid constituents of wheat flour have been shown by a number of workers to participate to a considerable extent in determining dough structure. The earlier literature on the role of lipids has been reviewed thoroughly by Cookson and Coppock (1), and a recent study has appeared by Smith, Van Buren, and Andrews (14) implicating the oxidation of polyunsaturated fatty acid components of the lipids in mixing properties of dough. Some evidence now shows that the lipids are involved in improver action. Moran, Pace, and McDermott (12) showed that application of nitrogen trichloride and chlorine dioxide to flour substantially increased the peroxide values of flour lipids. Cookson and Coppock (1) observed that that there was no very marked change in spectrum when the untreated flours (75–76% extraction) were treated with Agene or chlorine dioxide, although there was a noticeable increase in absorption in the region 210–240 $m\mu$ in the case of the lipid from chlorine dioxide-treated flour. Baking tests revealed that defatted untreated flour required less oxidative treatment to reach maximum loaf properties than natural flour, while defatted flour dough possessed greater resistance to extension and lower extensibility than doughs from natural flours. Cookson, Ritchie, and Coppock (2) confirmed these results in a second study and in addition showed that chlorine dioxide and bromate, although used in great excess, lowered the nitrogen and phosphorus content of the bread lipid. These results agreed with earlier reports (15) of Sullivan, Howe, Schmalz, and Astle-

[1] Manuscript received January 8, 1958. Paper No. 169 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba, and No. 345 of the Associate Committee on Grain Research (Canada).

ford, who found that lipid from bromated doughs contained less phosphorus and nitrogen than lipid from untreated dough.

Exploratory studies in this laboratory and evidence from the literature indicated that flour lipids were in some way involved in the decomposition of bromate in dough. Accordingly, the first objective of the experimental work described in this paper was to compare the amount of bromate decomposition in doughs made from undefatted flour, from flour partially defatted with petroleum ether, and flour wholly defatted with water-saturated n-butanol. Preliminary experiments had also shown that traces of oxygen (dissolved in the dough-mixing solutions) depressed the subsequent bromate reaction and that the depression was greater for doughs from defatted flours. Studies were therefore undertaken to determine the effect of mixing for increasing times, in nitrogen, air, and oxygen (representing 0, 20, and 100% oxygen) upon the decomposition of potassium bromate in doughs from normal and defatted flour. The results of these studies are presented.

Materials and Methods

The flour used for the main part of this study was a commercially milled untreated straight flour from hard red spring wheat. For supplementary studies, two commercial mill fractions were used. The designation and characteristics of these flours are given in Table I.

TABLE I
SPECIFICATIONS OF FLOURS USED IN THIS STUDY

Flour Stream	Petroleum Ether Extract	Water-Saturated n-Butanol Extract	Ash Content	Protein Content	Optimum Bromate
	%	%	%	%	*ppm*
Straight	1.04	2.31	0.50	13.1	10
Patent	0.77	1.93	0.38	11.6	5
Clear	2.24	3.87	1.02	18.5	25

Petroleum ether extract was determined by overnight extraction on Goldfisch extractors with petroleum ether (Skellysolve F 95); water-saturated n-butanol extract was determined by the method of Mecham and Mohammad (11). Ash and protein contents of the flours were determined by the methods of the AACC. Baking tests were made by the method of Geddes, Aitken, and Fisher (7).

Defatted flours were prepared by two methods. For removal of crude fat only, flour was extracted with petroleum ether (Skellysolve F 95) in a large Soxhlet extractor for several days. The flour was then

removed and spread out in shallow dishes to allow the solvent to evaporate. Finally, small samples of the flours were extracted overnight in Goldfisch extractors with Skellysolve F 95 to determine whether the flours were free of petroleum ether-soluble lipid. To effect complete lipid removal, flour was treated with deoxygenated, water-saturated n-butanol in the cold. After 16 hours (overnight) the n-butanol was filtered off and the flour was washed twice with cold, water-saturated ethyl ether. Residual ether was removed in a stream of nitrogen and the flour was left overnight exposed to an atmosphere of high humidity. The extracted lipids were recovered by careful evaporation *in vacuo* and were used for reconstitution experiments.

Doughs were mixed to 60% absorption at an initial level of 30 p.p.m. potassium bromate in the GRL mixer (8) under nitrogen, air, or oxygen. Flours to be mixed in nitrogen were pretreated by purging with nitrogen under alternate vacuum and pressure. The water used for mixing was used "as-is" for mixing in air but was saturated with either nitrogen or oxygen when mixing in these gases.

Bromate residues were determined (3) at hourly intervals for 5 hours and rate of bromate loss was taken as the slope of the line of best fit, as long as the rate of loss was linear. Average rate of loss, used as a measure of the amount of bromate decomposition, was derived from the slope of the line based on five separate analyses. This represents a considerable increase in accuracy over recoveries determined at a single time. In studies involving lower-grade flours, plotting recoveries against time also yields a visual indication of the linearity of the reaction.

Results

Effect of Defatting and Reconstituting upon Bromate Decomposition. Experiments described in this section were designed to establish a general relationship between flour lipids and the bromate reaction in dough. Rates of bromate loss were determined for the straight flour (No. 1 of Table I) undefatted, defatted with petroleum ether, and defatted with water-saturated n-butanol. Then flour defatted with petroleum ether was reconstituted with (a) 1.0% petroleum ether extract and (b) 1.0% n-butanol extract. Finally, n-butanol-extracted flour was reconstituted with 2.3% n-butanol extract and with 2.3 and 4.6% petroleum ether extract, and rates of bromate loss were again determined. All doughs were mixed in nitrogen only. The results are summarized in Table II.

The first three experiments show the effect of removal of flour

TABLE II

RATES OF BROMATE LOSS IN DEFATTED AND RECONSTITUTED FLOURS

FLOUR TREATMENT	BROMATE LOSS IN DOUGH
	ppm/hour
1. Undefatted	1.8
2. Petroleum ether-defatted	1.4
3. n-Butanol-defatted	0.4
4. Petroleum ether-defatted + petroleum ether extract	1.9
5. Petroleum ether-defatted + n-butanol extract	1.9
6. n-Butanol-defatted + n-butanol extract	0.4
7. n-Butanol-defatted + petroleum ether extract	0.4
8. n-Butanol-defatted + petroleum ether extract 2×	0.4

lipids on the rate of bromate reaction in dough. Removal of crude lipid (petroleum ether-extractable) depresses the rate of bromate loss from 1.8 to 1.4 p.p.m. per hour. Extraction with water-saturated n-butanol reduced the rate of bromate loss from 1.8 to 0.4 p.p.m. per hour, a considerably greater decrease than that caused by removal of crude lipid only.

The remaining five experiments listed in Table II were intended to answer the question whether the lipid reacted directly with bromate; or whether lipid acted as an intermediate between bromate and reducing materials in the flour. Direct reaction of extracted lipids with bromate presented experimental difficulties, so an alternate test was adopted. Flours defatted with either solvent were reconstituted with the recovered lipids, and subsequent rates of bromate loss in dough were again determined. For the petroleum ether-extracted flour, addition of petroleum ether extract or n-butanol-extracted lipid fully restored the original rate of bromate loss. On the other hand, neither n-butanol extract nor petroleum ether extract increased the bromate reaction with the n-butanol-extracted flour. Had either lipid fraction been capable of direct reaction with bromate, some increase in bromate decomposition was to be expected. Since no increase was found, lipid then appears to act only as an intermediate. Since removal of crude lipid depresses the bromate reaction reversibly, while removal of tightly bound lipid (i.e., n-butanol extract) causes irreversible loss of the bromate reaction, it may be inferred that two factors are involved. Furthermore, after complete extraction of the lipid by n-butanol the flour retains a residual reducing power towards bromate; substances other than lipid must remain in the flour to react with bromate.

Effect of Time of Mixing in Nitrogen, Air, and Oxygen on Rate of Bromate Reaction. The study of another aspect of the relation of flour lipids to bromate reaction was prompted by the finding that traces of

oxygen incorporated into dough with the mixing liquids substantially lowered the subsequent rate of bromate reaction in defatted flours. A systematic investigation was undertaken to study the effect of time of mixing in nitrogen, in air, and in oxygen upon the subsequent rate of bromate reaction in doughs from undefatted flours and from flours de-

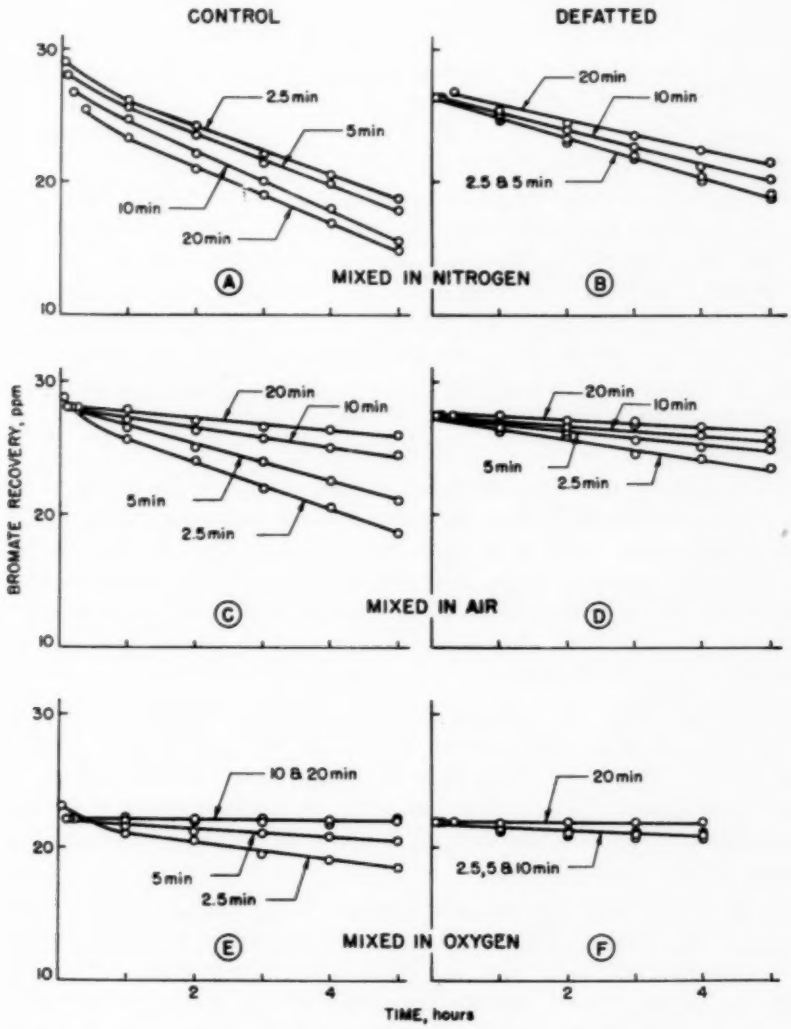

Fig. 1. Bromate recoveries in dough during 5 hours with initial level of 30 p.p.m. bromate. Control doughs: A, mixed in nitrogen; C, mixed in air; E, mixed in oxygen. Doughs from defatted flour: B, mixed in nitrogen; D, mixed in air; F, mixed in oxygen for 2.5, 5, 10, and 20 minutes.

fatted with petroleum ether. In this study, doughs were mixed in the GRL mixer for as long as 20 minutes; longer mixing results in the dough forming a viscous sheath on the walls of the mixer. Bromate was applied at the rate of 30 p.p.m. Bromate recoveries were determined at hourly intervals subsequent to each mixing time for each of the above conditions.

These primary data are summarized in Fig. 1 by plotting the recovery of bromate against reaction time in dough. The left half of Fig. 1 shows the effect of mixing in nitrogen, air, and oxygen upon the rate of bromate decomposition in a control flour; the right half of Fig. 1 shows the effect of these operations upon bromate loss in flour defatted with petroleum ether.

The first consequence to be drawn from these data is the effect of mixing alone (i.e., in nitrogen) upon bromate reaction. Figure 1, A, shows the effect upon subsequent bromate recoveries for the control mixed in nitrogen; Fig. 1, B, deals with recoveries for the defatted flour mixed in nitrogen. The changes in slope, although small, show that for the control flour, prolonged mixing increases the subsequent rate of bromate decomposition. For the defatted flour, on the other hand, prolonged mixing decreases the subsequent rate of bromate decomposition.

The second effect to be noted is the reduction in bromate decomposition effected by mixing in the presence of oxygen. Figure 1, C and E, shows the result of mixing the control flour in air and oxygen respectively. Figure 1, D and F, deals similarly with the defatted flour. Mixing in air or oxygen reduces the rate of bromate reaction and the amount of reduction is roughly proportional to the oxygen tension and the duration of mixing.

The final result of this study is that for a given mixing time doughs from defatted flours show a smaller bromate reaction, as evidenced by increased bromate recoveries, than do doughs from control flour. Mixing in air causes a large reduction of the bromate reaction of the defatted flour dough, while mixing in oxygen suppresses bromate decomposition almost entirely.

Figure 2 shows a further comparison of the foregoing data. Here the rates of decomposition of bromate, obtained as the slopes of the curves shown in Fig. 1, are plotted against mixing times of doughs. The results emphasize that mixing, especially in air or oxygen, predetermines to an important extent the rate of loss of bromate in dough. Removal of lipid appears to accentuate the role of oxygen as a competitive inhibitor of bromate decomposition.

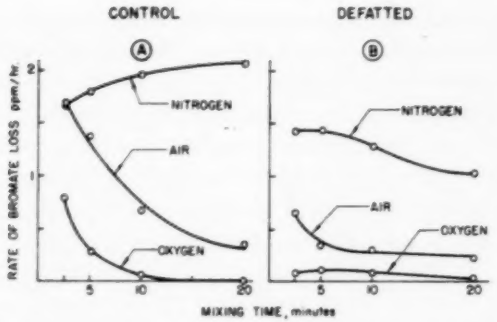

Fig. 2. Rates of bromate loss in dough plotted against mixing time for: A, control flour mixed in nitrogen, air, and oxygen; and B, defatted flour mixed in nitrogen, air, and oxygen.

Observations on Some Mill Fractions. In order to confirm the suggestion that doughs from defatted flours are more susceptible to the action of oxygen in depressing the rate of bromate reaction than doughs from ordinary flours, the rates of bromate decomposition were also determined for patent and clear (Nos. 2 and 3 of Table I), unextracted and defatted with petroleum ether and mixed for 5 minutes in nitrogen, air, and oxygen. These flours gave bromate reactions which could readily be expressed as linear rates of bromate loss in the usual way. They are given in Table III, with the straight flour (No. 1 of Table I) included for comparison.

TABLE III
RATES OF BROMATE LOSS FOR SEVERAL FLOURS
(p.p.m. per hour)

	Control			Defatted		
	Nitrogen	Air	Oxygen	Nitrogen	Air	Oxygen
Straight	1.79	1.37	0.27	1.44	0.24	0.06
Patent	1.85	0.68	0.21	0.75	0.56	0.37
Clear	3.08	2.70	0.19	2.72	1.09	0.35

In these examples, the clear flour resembles the straight but the patent flour differs in that the change in rate of bromate reaction upon defatting and mixing in nitrogen is very large, dropping from 1.85 to 0.75 p.p.m. per hour.

General Discussion

The effect of potassium bromate upon the physical properties of wheat flour doughs has been described by Dempster *et al.* (5,6) and by Hlynka and Anderson (9) as resulting from reactions which establish

new bonds between the macromolecules determining the structural properties of the dough. The progress of chemical reaction with bromate has necessarily been established by estimating residual bromate after a series of reaction times (3,4,10). While bromate has been shown to be consumed, no products of reaction, however, of bromate with dough constituents have ever been identified. This technique has not succeeded in relating the amount of bromate loss to changes in physical properties of dough; probably because the over-all rate of decomposition includes all the possible reactions of bromate with oxidizable material in the dough. Since only part of the over-all reaction may contribute to physical improvement (3,4), the aim of this study has been to identify the reactions taking place. Although earlier workers have implied a general relationship between lipid, mixing, oxygen, and improver action, the present work has served to focus attention on the effect of these factors on the chemical reaction of potassium bromate in dough. A fourth factor, previously established and confirmed in these studies, is the variation in flours found in mill fractions.

The effect of lipid, as determined by extraction and reconstitution, varied with the lipid fraction examined and was involved, as well, with the effects both of mixing and of oxygen. Removal of the petroleum ether-soluble fraction, presumably loosely bound lipids, from flour occasioned a small decrease in bromate decomposition in dough; reconstituting the flour restored the original rate of bromate decomposition. Removal of the n-butanol-soluble, or tightly bound lipids, occasioned a large and irreversible loss in bromate reaction. These facts suggested that the tightly bound lipids mediated between bromate and the reducing substance of dough. The nature of this mediation may reside partly in chemical transport of bromate oxidizing power, e.g., peroxide formation, and partly in the structural role of lipids in flour. Traub, Hutchinson, and Daniels (16), on the basis of X-ray analyses, suggested that the protein fibers of wheat flour are held together by layers of phospholipid, in the form of leaflets, with the fat molecules roughly perpendicular to the protein fibers. Since bromate action has been traditionally associated with protein, an association of lipid with protein might provide a basis for lipid mediation in the bromate reaction.

Mixing in nitrogen tends to increase the rate of subsequent bromate reaction for control flours and to decrease the rate for defatted flours. This constitutes support for the hypothesis of the intermediary role of lipid in the bromate reaction, for continuous mixing to more

intimate association of constituents would promote reaction with bromate. On the other hand, in the absence of lipid-bridging material, increased mixing might result in increased adhesion between protein fibers, with corresponding "burial" of reducing groups and lowered bromate reaction.

The effect of mixing in air or oxygen is more straightforward. Oxygen is incorporated into the dough and reacts preferentially to bromate with reducing matter. The reduction in rate of bromate reaction is roughly proportional to oxygen tension and amount of mixing. This competitive reaction of oxygen to bromate supports, in a chemical sense, the conclusions of Dempster, Hlynka, and Anderson (5) and Smith and Andrews (13) drawn from physical tests, that the improving action of oxygen is similar to that of bromate.

A second effect of oxygen is the larger reduction in bromate reaction which takes place in defatted as compared to control doughs when they are mixed in air or oxygen. This suggests that the residual reducing matter in flour is more susceptible to oxygen attack in defatted than in control flours, possibly because natural antioxidants, e.g. vitamin E, have been removed in defatting.

This augmented oxygen attack upon defatted flours offers an explanation of the contradictory reports of the effect of lipid extraction upon subsequent baking quality. It is simply this: some defatted flours may incorporate enough oxygen in mixing to effect overimprovement with consequent reduction in loaf volume; this might be interpreted as damage to flour quality. For other flours, only enough oxygen might be incorporated to effect improvement with increase in loaf volume. The amounts of oxygen incorporated would vary with mixing time and type of mixer used and probably, as well, with the flour, so that conflicting results might easily arise.

These studies have only partially succeeded in the original purpose of identifying specific reactions of potassium bromate with dough constituents; several factors implicating the lipids in the reaction have been demonstrated. Elucidation of these factors confirms previous hypotheses (3,4) that the reaction of potassium bromate in doughs is complex; further studies in this direction may yet serve to relate specific chemical reactions to changes in the physical properties of dough.

Acknowledgment

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THE DISTRIBUTION OF AMINO ACIDS IN WHEAT AND CERTAIN WHEAT PRODUCTS[1]

MILLARD J. HORN, COLBURN C. FIFIELD, AMOS E. BLUM, AND HELEN W. WARREN

ABSTRACT

Eleven amino acids of whole wheat were quantitatively accounted for when the extraction products, flour, bran, and shorts were assayed by microbiological procedures. No destruction of amino acids occurred during fermentation of the doughs prior to baking into bread. The standard deviation of the amino acid values for all products was calculated, and an analysis of variance was applied to ascertain significant differences between the mean amino acid values for doughs and breads. Losses of cystine, lysine, and methionine during baking were significant. Most of these amino acid losses may have taken place in the crusts. Since small experimental loaves were used in these experiments and since the proportion of crust to crumb is greater than in commercial loaves, it is probable that in the analysis of commercial loaves, some of the losses of amino acids may not be significant.

Extensive investigations have been carried on in an effort to improve the agronomic, milling, and baking quality of wheat. Little or no attention has been given to the distribution of amino acids in the flours and in the feed fractions from the wheat nor to the retention or loss of amino acids in yeast-leavened or unleavened doughs and breads.

The experiments reported in this paper were designed for a multiple purpose: 1), to obtain information on the amino acid distribution in wheat and wheat products; namely, two flours (71.5% and 84.9% extraction), the bran and shorts from these extractions, and breads prepared from these flours; 2), to determine the nature and extent of amino acid losses, if any, during various stages of processing; and 3), to test further the reliability and precision of the methods developed in this laboratory (3,4,5,8).

Materials and Methods

Preparation of Wheat and Wheat Products. The experimental work was divided into two phases. In the first phase a hard red winter wheat which had a test weight per bushel of 61.3 pounds and a protein content of 12.3% (14.0% moisture basis) was experimentally milled on the Buhler automatic mill. One portion of the wheat was milled to produce a straight grade flour of 71.5% extraction (used in bread A) and produced 16.9% bran and 7.9% shorts with an overall milling loss of

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3.7%.[2] Another portion of the wheat was milled to an 84.9% extraction flour (used in bread B) and produced 0.9% bran[3] and 12.1% shorts, with an overall loss of 2.1%.[4] For the bread-baking tests, breads A and B were made by a formula described by Fifield *et al.* (2), using 100 g. of flour, 2.0 g. of compressed yeast, 1.5 g. of salt, 5.0 g. of sugar, 3.0 g. of shortening, and 4.0 g. of nonfat dry milk. The doughs after mixing were fermented for 3 hours at 86°F. (30°C.), panned and proofed for 55 minutes at 86°F. (30°C.), and then baked for 25 minutes at 450°F. (232°C.).

In the second phase of this study, a commercially milled, good-quality, bread wheat flour was used which had a protein content of 12.5% and an ash content of 0.50% (both on a 14.0% moisture basis). Two breads were made from this flour by the standard procedure (2): bread C from yeast-leavened dough, bread D from unleavened dough. Nitrogen determinations were made after each step in processing.

The wheat and wheat products were assayed microbiologically for eleven amino acids using methods published previously (3,4,5,8). Samples of wheat and wheat products which contained approximately 100 mg. of protein were used for hydrolysis for all assays. The amino acid contents of the ingredients used in preparing the bread doughs were determined, and the amounts that should be present in breads A and B were calculated. Breads A and B were assayed for their amino acid content and these values compared with the calculated values at the same nitrogen level. The breads and doughs (C and D) prepared in the second part of this study were also assayed for eleven amino acids, and the values obtained for the doughs were compared with those found in the corresponding breads.

Results and Discussion

Table I shows the nitrogen content of the wheat, flours, doughs, breads, brans, and shorts used in the experiments. All values for doughs and breads were calculated to a 1.45% total nitrogen basis in order that they could be compared with each other. The composition of the four doughs that were made into breads was different. Doughs A and B differed in the composition of the flour. Doughs C and D differed because no yeast was added to dough D. Flours of similar extraction were used in doughs A, C, and D, but the nitrogen content of flour A was different from C and D.

[2] These losses were assumed to be distributed in the different fractions in the same proportions as their respective yields.

[3] The type of mill used causes some of the bran to be carried over into the "shorts" fraction. Other types of mills with finer screens would give a larger bran fraction.

[4] See footnote 2.

TABLE I

NITROGEN CONTENT OF SAMPLES

SERIES	WHOLE WHEAT	FLOURS	DOUGHS	BREADS[a]	BRAN	SHORTS
	%	%	%	%	%	%
A	2.19	2.02	2.16[b]	2.12	2.57	2.82
B	2.19	2.20	2.33[b]	1.91	1.73	2.28
C	...	2.22	1.45	2.54	...	...
D	...	2.22	1.42	2.35	...	...

[a] Some breads were dried in order to obtain a homogeneous mixture of crust and crumb.
[b] 106 g. contained 2.291 g. nitrogen and 2.471 g. nitrogen, respectively.

TABLE II

AMINO ACID CONTENT OF THE INGREDIENTS ADDED TO FLOUR

AMINO ACID	NONFAT DRY MILK 5.51% N	BAKER'S COMPRESSED YEAST 2.53% N
	g/100 g	*g/100 g*
Arginine	1.37	0.74
Cystine	0.34	0.12
Histidine	0.96	0.32
Isoleucine	2.25	0.89
Leucine	3.35	1.07
Lysine	2.95	1.22
Methionine	0.97	0.24
Phenylalanine	1.96	0.73
Threonine	1.56	0.76
Tyrosine	1.69	0.56
Valine	2.43	0.88

Table II gives the amino acid content of the ingredients added to flour.

Table III gives, for each of eleven amino acids, the mean and standard deviation of the flours, brans, and shorts assayed in this experiment, and Table IV gives the same information for the breads and doughs.

Amino Acid Distribution in Wheat Products. Table V shows the amino acid content of wheat and wheat products from a 71.5% and 84.9% flour extraction and the changes in amino acid content of the wheat products caused by the different extractions. Comparison of the amino acids of the two flours of Table V shows increases of all amino acids in the flour obtained from the 84.9% extraction. Lysine was increased 32%, methionine 17%, and the other amino acids were increased from 9 to 15%. It also shows the reconciliation of the amino acids in bran, shorts, and flour with the corresponding amino acids in whole wheat.

Table VI shows the change in amino acid content of the dough caused by the added ingredients, milk and yeast. The values of doughs

TABLE III

AMINO ACIDS IN FLOURS, BRANS, AND SHORTS
(Grams of amino acids per 100 g. of product)

AMINO ACID	A						B					
	FLOUR		BRAN		SHORTS		FLOUR		BRAN		SHORTS	
	Mean[a]	S[b]	Mean	S	Mean	S	Mean	S	Mean	S	Mean	S
	%	%	%	%	%	%	%	%	%	%	%	%
Arginine	0.505	0.018	1.11	0.022	1.23	0.047	0.573	0.013	0.76	0.022	1.00	0.009
Cystine	.297	.010	0.29	.013	0.38	.020	.338	.008	.24	.008	0.32	.010
Histidine	.270	.008	.44	.010	0.45	.013	.298	.005	.29	.020	.38	.013
Isoleucine	.599	.021	.64	.025	0.75	.029	.648	.004	.40	.000	.53	.022
Leucine	.842	.016	.95	.017	1.06	.028	.967	.017	.62	.040	.83	.044
Lysine	.274	.016	.64	.031	0.77	.017	.371	.006	.42	.008	.59	.008
Methionine	.177	.006	.21	.010	0.24	.006	.208	.007	.13	.006	.18	.008
Phenylalanine	.677	.011	.72	.010	0.78	.026	.766	.043	.48	.008	.62	.028
Threonine	.368	.014	.55	.019	0.63	.022	.410	.022	.37	.022	.47	.021
Tyrosine	.318	.014	.37	.021	0.44	.012	.349	.019	.26	.008	.35	.006
Valine	0.532	0.009	0.73	0.028	0.83	0.016	0.604	0.023	0.51	0.008	0.68	0.006

[a] Each mean is based on four values.
[b] Standard deviation.

TABLE IV

Amino Acids in Doughs and Breads
(Calculated to 1.45% N)

Amino Acid	A				B				C				D			
	Dough		Bread		Dough		Bread		Dough		Bread		Dough		Bread	
	Mean[a]	S[b]	Mean	S	Mean	S	Mean	S	Mean	S	Mean	S	Mean	S	Mean	S
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Arginine	0.364	0.012	0.341	0.011	0.378	0.007	0.370	0.015	0.329	0.005	0.303	0.009	0.333	0.012	0.332	0.026
Cystine	.198	.006	.191	.004	.208	.004	.192	.008	.215	.003	.198	.003	.217	.004	.222	.012
Histidine	.199	.005	.192	.004	.201	.004	.189	.005	.196	.004	.197	.001	.202	.004	.206	.001
Isoleucine	.448	.011	.416	.019	.444	.004	.415	.027	.394	.020	.424	.014	.412	.022	.421	.014
Leucine	.632	.011	.622	.008	.658	.011	.618	.011	.640	.013	.683	.008	.667	.017	.688	.009
Lysine	.263	.012	.222	.005	.301	.004	.233	.007	.262	.006	.216	.010	.243	.000	.209	.003
Methionine	.140	.004	.126	.005	.147	.003	.131	.005	.136	.003	.116	.004	.142	.004	.142	.003
Phenylalanine	.487	.005	.456	.021	.504	.028	.456	.007	.482	.018	.511	.012	.474	.006	.507	.018
Threonine	.282	.008	.272	.007	.284	.014	.280	.004	.264	.009	.284	.005	.260	.006	.278	.011
Tyrosine	.251	.010	.198	.006	.251	.010	.200	.006	.218	.005	.207	.005	.220	.010	.193	.002
Valine	0.409	0.006	0.425	0.023	0.422	0.014	0.417	0.026	0.410	0.023	0.426	0.015	0.417	0.026	0.423	0.020

[a] Each mean is based on four values.
[b] Standard deviation.

TABLE V

AMINO ACID CONTENT OF WHEAT PRODUCTS AND RECONCILIATION WITH THE WHEAT AFTER A 71.5% AND AN 84.9% FLOUR EXTRACTION
(Grams per 100 grams)

AMINO ACID	PRODUCTS FROM 71.5% FLOUR EXTRACTION			PRODUCTS FROM 84.9% FLOUR EXTRACTION			WHOLE WHEAT		
	Bran (16.9%)	Shorts (7.9%)	Flour (71.5%)	Bran (0.9%)	Shorts (12.1%)	Flour (84.9%)	From 71.5% Extraction Products[a]	From 84.9% Extraction Products[b]	Whole Wheat Assay
Arginine	1.11	1.23	0.51	0.76	1.00	0.57	0.67	0.63	0.63
Cystine	0.29	0.38	.30	.24	0.32	.34	.31	.34	.33
Histidine	0.44	0.45	.27	.29	0.38	.30	.31	.31	.32
Isoleucine	0.64	0.75	.59	.40	0.53	.65	.61	.63	.62
Leucine	0.95	1.06	.84	.62	0.83	.97	.88	.94	.90
Lysine	0.64	0.77	.28	.42	0.59	.37	.37	.39	.38
Methionine	0.21	0.24	.18	.13	0.18	.21	.19	.21	.20
Phenylalanine	0.72	0.78	.68	.48	0.62	.77	.70	.75	.71
Threonine	0.55	0.63	.37	.37	0.47	.41	.42	.42	.43
Tyrosine	0.37	0.44	.32	.26	0.35	.35	.34	.35	.36
Valine	0.73	0.83	0.53	0.51	0.68	0.61	0.59	0.62	0.59

[a] Correction made for 3.7% loss during milling.
[b] Correction made for 2.1% loss during milling.

A and B are, of course, calculated. The increase of amino acids effected by the addition of milk and yeast compared with flour alone is considerable in some cases. For example, the lysine content was increased 52% in dough A and 38% in dough B; the methionine content 25% in dough A and 21% in dough B. Since the dough A and dough C were the same except for a small difference in total nitrogen of the flours, it was interesting to compare the lysine and methionine content of dough A, which was calculated, and dough C, which was actually analyzed. The calculated values for lysine and methionine in dough A (Table VI) were 0.263 and 0.140, respectively; whereas the values for dough C (Table IV) were 0.262 and 0.136.

TABLE VI

CALCULATED AMINO ACID DISTRIBUTION IN DOUGHS A AND B

Amino Acid	Dough A				Dough B			
	100 g[a] Flour	4 g[a] Nonfat Dry Milk	2 g[a] Yeast	Calculated[b] Value 1.45% N	100 g[a] Flour	4 g[a] Nonfat Dry Milk	2 g[a] Yeast	Calculated[b] Value 1.45% N
	g	*g*	*g*	*g/100 g*	*g*	*g*	*g*	*g/100 g*
Arginine	0.505	0.055	0.015	0.364	0.573	0.055	0.015	0.378
Cystine	.297	.014	.002	.198	.338	.014	.002	.208
Histidine	.270	.038	.006	.199	.298	.038	.006	.201
Isoleucine	.599	.090	.018	.448	.648	.090	.018	.444
Leucine	.842	.134	.021	.632	.967	.134	.021	.658
Lysine	.274	.118	.024	.263	.371	.118	.024	.301
Methionine	.177	.039	.005	.140	.208	.039	.005	.147
Phenylalanine	.677	.078	.015	.487	.766	.078	.015	.504
Threonine	.368	.062	.015	.282	.410	.062	.015	.284
Tyrosine	.318	.068	.011	.251	.349	.068	.011	.251
Valine	0.532	0.097	0.018	0.409	0.604	0.097	0.018	0.422

[a] Based on four values.
[b] Nitrogen for A = 2.291, for B = 2.471.

Results from the first phase of this study indicated the possibility that the amino acid changes might have occurred during fermentation rather than during baking. In order to determine if this were the case, two doughs were prepared (C and D), with and without yeast and analyzed for the amino acids directly after mixing and after fermentation. The results, together with the mean squares obtained from an analysis of variance applied to the data, are given in Table VII. Of the twenty-two assays, only two differences were significant: threonine for dough C and phenylalanine for dough D. Since these differences were increases instead of losses, they are probably due to error in assay and are therefore discounted. These results indicate that there is no loss of amino acids during fermentation.

Table VIII shows mean squares obtained from an analysis of variance applied to the pooled data for doughs and breads. It is assumed

TABLE VII

MEANS AND MEAN SQUARES FOR DOUGHS C AND D, BEFORE AND AFTER FERMENTATION

(Calculated to 1.45% N)

AMINO ACID	MEANS[a]		MEAN SQUARE × 10^{-6}		MEANS[a]		MEAN SQUARE × 10^{-6}	
	C[b]	C'[b]	Within Doughs	Between Treatments[b]	D[b]	D'[b]	Within Doughs	Between Treatments[b]
	%	%			%	%		
Arginine	0.329	0.332	69	24	0.333	0.335	94	6
Cystine	.215	.222	24	78	.217	.222	21	50
Histidine	.196	.196	27	2	.202	.205	15	21
Isoleucine	.394	.405	270	253	.412	.404	341	144
Leucine	.640	.648	157	98	.667	.676	181	162
Lysine	.262	.266	25	28	.243	.241	38	8
Methionine	.136	.136	9	0	.142	.145	48	10
Phenylalanine	.482	.493	306	242	.474	.490	72	512*
Threonine	.264	.280	45	512*	.260	.267	38	91
Tyrosine	.218	.222	6	32	.220	.214	86	85
Valine	0.410	0.429	342	722	0.417	0.430	1052	351

[a] Each mean is based on four values.

[b] C and D before fermentation; C' and D' after fermentation. Treatments are designated as before and after fermentation.

* Mean square significantly higher than mean square for within doughs at 5% level.

that the doughs and breads can be assayed with the same precision. The mean values for the amino acids were not the same in the various doughs since the doughs differed in composition, and these differences should be reflected in the breads. Significant differences between mean values for amino acids of the doughs and breads are indicated by mean squares which are starred. These are arginine, cystine, histidine, lysine, methionine, and tyrosine.

TABLE VIII

MEAN SQUARES FOR POOLED DATA ON FOUR DOUGHS AND FOUR BREADS

AMINO ACID	WITHIN DOUGHS AND BREADS 10^{-6}	BETWEEN DOUGHS 10^{-6}	BETWEEN BREADS 10^{-6}	DOUGH VS. BREAD 10^{-6}
Arginine	181	2227**	3027**	1667**
Cystine	39	292**	846**	631**
Histidine	15	34	206**	101*
Isoleucine	311	2723**	70	248
Leucine	129	1044**	5704**	86
Lysine	47	2383**	416**	10981**
Methionine	16	82**	453**	1301**
Phenylalanine	266	650	3828**	144
Threonine	74	622**	89	300
Tyrosine	51	1360**	136	10153**
Valine	410	137	68	569

* Mean square significantly higher than mean square for within doughs and breads at 5% level.
** Mean squares significantly higher than mean square for within doughs and breads at 1% level.

Evaluation of Results. A critical evaluation of the results of these experiments depends primarily on the adequacy of the analytical methods used to assay the wheat and wheat products. The presence of carbohydrates in varied amounts and the small amounts of protein compared to other materials in the wheat make it imperative that the methods used be accurate enough to evaluate the results intelligently. In the first experiment the analysis of wheat and wheat products (Table V) containing different amounts of protein and other materials afforded a good opportunity to test the suitability of the methods used. The standard deviations found in assaying the amino acids of the bran, shorts, flour, and wheat were found to be satisfactory with an average coefficient of variation for all amino acids less than 5% (Table III). However, the results do not show whether amino acids were destroyed during hydrolysis. If the sum of one amino acid of the three hydrolysates (bran, shorts, flour) for the 71.5% flour extraction equals the amino acid content of the one hydrolysate (wheat), it can be from only one of two things: either the total destruction in the three hydrolysates for each amino acid is equal to the destruction of the same amino acid in one hydrolysate, or there is no destruction at all under the conditions

used. It is true that a loss of 20% for an amino acid in the assay of bran would reflect a loss of 2 or 3% in the total, but losses of 20% in hydrolyzing flour would show considerable loss in the total. If random destruction were occurring during hydrolysis of the various wheat fractions, the same degree of destruction could not be assumed. Therefore, the sum of each amino acid of the three hydrolysates would not always agree with the corresponding amino acid value of the whole wheat. The same argument applies to the 84.9% extraction where the flour, bran, and shorts have a different composition. Thus, the conclusion is that the methods used have a precision which makes possible reasonable deductions of losses or gains in amino acids.

Table VIII shows the differences in amino acid content of the different doughs and breads and the overall loss of amino acids between the pooled doughs and pooled breads. Of the six significant differences, it is believed that the losses of arginine and histidine may be discounted and may average out as more samples are assayed. The losses of cystine, lysine, and methionine, however, cannot be ignored and must be considered losses in baking. The significance of the tyrosine value is open to doubt since tyrosine is lost on acid hydrolysis in the presence of sugar. This is shown in Table IV, where the calculated values of tyrosine in doughs A and B are shown to be much higher than in doughs C and D which were actually assayed and further the close agreement of tyrosine values for breads A, B, C, and D (Table IV). A large part of the loss was because of the differences between the calculated values for doughs A and B and assay values for breads A and B.

The significant losses of amino acids may be explained by the unusually large ratio of crust to crumb in the small experimental loaves assayed. Analyses of larger commercial loaves, where the ratio of crust to crumb is smaller, might reduce the significance of the losses of amino acids. Analyses of cottonseed cooked at different temperatures and pressures and different lengths of time have shown no destruction of amino acids unless the protein is destroyed by excessive temperatures (1,6,7). These results have been confirmed in this laboratory on about 40 foods containing either animal or vegetable proteins (unpublished data). Analyses of breads baked under commercial conditions would be required in order to determine whether losses in commercial breads are significant.

This paper deals only with the amino acid *contents* of breads as determined on acid hydrolysates. It does not indicate to what extent nor which amino acids may be made physiologically unavailable for nutritional purposes by the process of baking. This "tying-up" or

"binding" effect has been shown in both published and unpublished reports of work done in this and many other laboratories on various foods. In view of this fact, a study of the amino acid availability from the standpoint of nutrition may change the concept of the value of the amino acid distribution as shown by acid hydrolysates.

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THE DISTRIBUTION OF LIPASE IN THE COMMERCIAL MILL PRODUCTS FROM HARD RED SPRING WHEAT[1]

MADELYN FERRIGAN AND W. F. GEDDES

ABSTRACT

Lipase activity was determined on all 32 commercial mill streams of a hard red spring wheat mix by measuring the glycerol liberated from a monoolein substrate in 1 hour at 30°C. and pH 7.35. The second sizings stream contained the lowest lipase activity, 12 γ glycerol released per g. flour, and the No. 1 germ stream contained the highest activity, 2354 γ glycerol per g. When the wheat extraction was progressively increased by accumulating the various mill products in order of increasing ash content, the distribution curves for lipase and ash were superimposable. The 27 flour streams representing 73.5% of the total yield contained only 21.3% of the total lipase and total ash in contrast with 69.8% of the total protein and 44.3% of the total lipid.

Simple correlations between lipase activity of the various flour streams and their protein, ash, and lipid contents were +0.59, +0.88, and +0.60 respectively. Although highly significant, these associations are not sufficiently close to provide an accurate estimate of lipase activity from a knowledge of protein, ash, or lipid content.

The most pronounced chemical change which has been observed during the deterioration of wheat and wheat products upon storage is an increase in fat acidity. It is therefore rather surprising that little information is available concerning the wheat lipases or their distribution in the wheat kernel (5). However, most procedures for measuring lipase activity have depended upon the measurement of fatty acids released during lipid hydrolysis, and they are not sufficiently sensitive for accurate determination of the low activities of highly refined flours. Recently, in these laboratories, Luchsinger *et al.* (3) developed a sensitive assay procedure based upon the estimation of glycerol released from a monoolein emulsion. They applied this procedure to the analysis of three grades of flour and to four feed fractions from hard red spring wheat. The results confirmed the earlier observations of Engel (2) and of Miller and Kummerow (4) that wheat germ had the highest activity, whereas endosperm or patent flour had very low activity.

This new method has been applied to a study of the lipase activity of all the mill streams from hard red spring wheat. The relation be-

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tween its distribution and that of the ash constituents, the proteins, and the lipids was also ascertained.

Materials and Methods

Samples of all the products obtained in the milling of a hard red spring wheat mix were procured from a large commercial mill. These consisted of 27 flour and 5 feed streams; information was provided on the percentage of the entire wheat represented by each stream. The feed streams were ground in a micro-Wiley mill to pass a No. 40 sieve; all samples were well mixed and stored at about −4°C. until analyzed.

The moisture, protein, and ash content of the samples were determined according to the standard procedures outlined in *Cereal Laboratory Methods* (1). Lipid content was determined by extracting 2.0 g. of the moisture-free samples with 175 ml. Skellysolve B in a Soxhlet for 4 to 6 hours, evaporating the solvent under vacuum on a water bath maintained at 45° to 50°C., cooling in a desiccator, and weighing the residue.

For the lipase activity determinations, the bulk of the lipid was extracted from 200-g. portions of each sample by five successive extractions with Skellysolve B, using a total volume of about 800 ml.; and the samples were allowed to air-dry at room temperature. Lipase activity was assayed by a slight modification of the method of Luchsinger *et al.* (3) which involves determining the amount of glycerol liberated from a monoolein[2] emulsion in 1 hour at 30°C. and pH 7.35. After inactivation of the enzyme and destruction of the emulsion with trichloroacetic acid and calcium chloride, the glycerol in the aqueous phase is determined by periodate cleavage, reaction of the resulting formaldehyde with chromotropic acid, and measurement of the absorbance of the colored complex at 570 mμ. The lipase activity is expressed as γ of glycerol liberated from the monoolein substrate per g. of original sample at 14.0% moisture.

In preliminary trials, rather wide variations were obtained in the absorbance readings for replicate assays of flour samples. These were traced to the difficulty of obtaining an aliquot of the aqueous layer which was entirely free from flour particles, and the following modifications of the assay procedure described by Luchsinger *et al.* were adopted. After the first centrifugation following addition of Skellysolve to the inactivated reaction mixture, the aqueous layer and supernatant Skellysolve were carefully decanted into another test tube. The flour particles which collected into a compact layer at the interface of

[2] Commercial monoolein (Glyco Products Inc., Brooklyn, N.Y.), which contains 1-monoolein, 1,1'-diolein, and a trace of triolein, was employed.

the two solvents during centrifugation remain in the first tube if it is rotated carefully during decantation. The aqueous layer was then emulsified with Skellysolve B by vigorous shaking, after which the

TABLE I

PROTEIN, ASH, LIPID CONTENT AND LIPASE ACTIVITIES OF MILL STREAMS OBTAINED IN THE COMMERCIAL MILLING OF HARD RED SPRING WHEAT
(Data are expressed on a 14.0% moisture basis)

MILL STREAM	PROTEIN	ASH	LIPID[a]	LIPASE[b] ACTIVITY
	%	%	%	γ/g
Break flour				
Pre-break	15.0	0.68	1.8	88
First and second break	15.5	0.50	1.1	92
Third break	17.8	0.68	3.3	115
Fourth break	19.2	1.11	2.7	211
Middlings flours				
First	11.7	0.37	1.0	52
Second	11.2	0.35	1.2	47
Third	11.3	0.33	1.4	55
Fourth	12.7	0.37	1.3	56
Fifth	12.7	0.41	1.3	95
Sixth	12.7	0.48	1.3	78
Seventh	12.7	0.46	1.1	123
Eighth	11.9	0.56	1.2	101
Ninth	12.1	0.74	1.3	205
Tenth	15.8	1.12	2.3	269
Eleventh	15.5	1.35	2.6	232
Twelfth	14.4	1.57	2.5	333
Sizings flours				
Fines	12.1	0.38	1.0	84
First	12.5	0.49	1.9	71
Second	12.1	0.39	1.3	12
Third	11.9	0.59	1.9	55
Miscellaneous flours				
First tailings	11.6	0.42	2.3	128
Second tailings	12.3	0.52	1.7	128
Third tailings	12.5	0.56	1.7	83
Fourth tailings	14.0	0.98	2.6	172
Bran dust	20.5	1.32	1.4	257
Poor suction	15.4	1.02	3.3	312
Middlings suction	13.0	0.47	0.9	129
Feed streams				
Red dog	15.9	3.19	3.7	1049
Shorts	16.0	4.88	4.8	883
First germ	27.9	4.54	10.9	2354
Second germ	22.8	4.65	8.9	2100
Bran	14.1	6.12	4.9	953
Whole wheat	14.2	2.20	2.4	434
All streams				
Minimum	11.2	0.33	0.9	12
Maximum	27.2	6.12	10.9	2354
Mean	14.6	1.30	2.5	341
Standard deviation	3.6	1.5	2.2	550
Coefficient of variation	24.7	118.1	87.2	161.2

[a] Determined by extraction with Skellysolve B.
[b] Lipase is expressed as γ of glycerol liberated from monoolein per g. of original sample.

corked tubes were centrifuged at 3°C. for 20 minutes. The colorimetric determination was made on 1 ml. of the aqueous phase.

Results and Discussion

The analytical results for each sample are recorded in Table I. The lipase activities ranged from 12 units for the second sizings stream to 2354 units for the No. 1 germ stream. The standard error based on duplicate determinations was 21.7 units, or 6.4% in terms of the mean activity for all samples. A composite sample representing all mill streams in proportion to their respective yields contained the same lipase activity as the original wheat.

Correlation coefficients and regression equations for the relation between lipase activity and the protein, ash, and lipid contents of the 27 flour streams, together with the standard errors of estimate for individual flours, are given in Table II. The highly significant positive correlations between lipase activity and these variables are not sufficiently great to permit its accurate prediction from a knowledge of the protein, ash, or lipid contents.

TABLE II

RELATION BETWEEN LIPASE ACTIVITY AND THE PROTEIN, ASH, AND LIPID CONTENTS OF THE TWENTY-SEVEN FLOUR STREAMS

FACTORS	CORRELATION COEFFICIENT [a]	REGRESSION EQUATION	STANDARD ERROR OF ESTIMATE [b]
Lipase activity (L) × Protein content (P)	+0.59	L = 20.8 P − 152.2	72.1
Lipase activity (L) × Ash content (A)	+0.88	L = 207.0 A − 6.1	39.5
Lipase activity (L) × Lipid content (F)	+0.60	L = 72.6 F − 266.7	67.1

[a] Value of r at 1% point = 0.49.
[b] For individual flours.

Since the yield of each stream as a percentage of the wheat was available, it was possible to compute weighted mean analytical values for selected groups of flour streams and for the feed streams. These data are recorded in Table III. Among the flour streams, the sizings flours had the lowest lipase activity, ash and lipid content, while the break flours gave the highest values for these constituents as well as for protein content. All the flour streams combined had a weighted mean activity of 90.4 lipase units in contrast to 926.5 units for all the feed streams.

The distribution of the total protein, ash, lipid, and lipase contents

TABLE III

WEIGHTED MEAN VALUES FOR SELECTED GROUPS OF STREAMS[a]

WHEAT FRACTION	NUMBER OF STREAMS	MILL YIELD	PROTEIN	ASH	LIPID	LIPASE[b] ACTIVITY
		%	%	%	%	γ/g
Break flours	4	14.4	16.5	0.63	1.8	113.6
Middlings flours	12	35.5	12.0	0.44	1.3	78.0
Sizings flours	4	16.8	12.1	0.40	1.2	68.6
Miscellaneous flours	7	6.8	14.0	0.68	1.7	159.4
All flours	27	73.5	13.1	0.49	1.4	90.4
Feed streams	5	26.5	15.7	5.04	4.8	926.5

[a] All analytical data are expressed on 14.0% moisture basis.
[b] Lipase activity is expressed as γ of glycerol liberated from monoolein per g. of original sample.

of the wheat which are present in different composite fractions is shown in Table IV. To show the effect of extraction on the distribution of these constituents in greater detail, the various mill streams were accumulated in order of increasing ash content. The composition of each successively larger pooled fraction was computed and related to the total for the wheat. These percentage distributions of the protein, lipid, ash, and lipase activity are plotted against the percentage extractions in Fig. 1.

TABLE IV

DISTRIBUTION OF TOTAL PROTEIN, ASH, LIPID, AND LIPASE CONTENTS IN DIFFERENT COMPOSITE FRACTIONS

WHEAT FRACTION	NUMBER OF STREAMS	MILL YIELD	PROTEIN	ASH	LIPID	LIPASE ACTIVITY
			%	%	%	
Break flours	4	14.4	17.2	5.3	11.2	5.2
Middlings flours	12	35.5	31.0	9.2	19.7	8.9
Sizings flours	4	16.8	14.7	3.9	8.5	3.7
Miscellaneous flours	7	6.8	6.9	2.8	4.9	3.5
All flours	27	73.5	69.8	21.3	44.3	21.3
Feed streams	5	26.5	30.2	78.7	55.7	78.7
Total	32	100.0	100.0	100.0	100.0	100.0

The flour streams contained more of the total protein and total lipids than was the case for either lipase or ash content. The distributions of ash and lipase activity were very similar. The 27 flour streams representing 73.5% of the wheat contained only 21.3% of the total lipase and total ash in contrast to 69.8% of the total protein and 44.3% of the total lipid. The feed streams representing only 26.5% of the wheat contained 78.7% of the total ash and total lipase. Indeed, the shorts representing 19.0% of the total yield contained 53.6% of the total lipase.

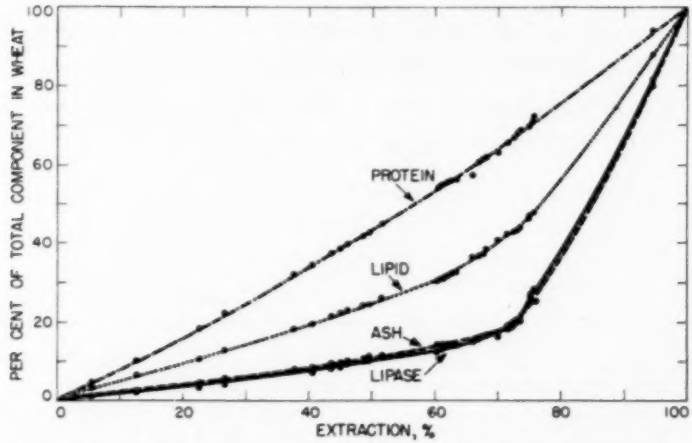

Fig. 1. Relations between percent extraction and the percentage of the total protein, lipid, ash, and lipase concentrations represented by the composite fractions accumulated in order of increasing ash content.

If it is assumed that the wheat contained 2.3% germ and that the germ which was not isolated as such (amounting to 1.9%) is all in the shorts fraction, the contribution of the germ and germ-free shorts to the total lipase activity can be computed. These calculations show that the estimated germ content of 2.3% would contribute 15.9% of the total lipase activity of the wheat. The germ-free shorts would have an activity of 126.5 lipase units which represents 40.5% of the total activity. It is clear that certain wheat tissues, in addition to the germ proper, are of very high lipase activity.

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COOKING QUALITY OF WHITE RICE MILLED FROM ROUGH RICE DRIED AT DIFFERENT TEMPERATURES[1]

OLIVE M. BATCHER,[2] RUBY R. LITTLE,[2] ELSIE H. DAWSON,[2] AND JOSEPH T. HOGAN[3]

ABSTRACT

Quality evaluations were made on samples of Century Patna and Rexoro varieties of rice which had been air-dried at room temperature or forced-air dried at 120°F. (49°C.), 140°F. (60°C.), or 160°F. (71°C.). A panel evaluated color, cohesiveness, and absence or presence of off-flavor of the cooked rice by ranking and scoring techniques. Measurements were made of volume, water absorption, starch, and total solids after treatment with water at 99°C., and also of the reaction of the rice to treatment with dilute alkali.

Variations in some of the quality characteristics by panel evaluations, and by treatment with water at 99°C. or with dilute alkali were observed for some of the milled samples of rough rice dried at different temperatures. However, the variations were not linearly related to successive increases in temperatures, nor were they similar for the two varieties. Forced-air drying of rough rice at elevated temperatures did not cause marked improvement or deterioration in the cooking quality of milled rice.

Heat is usually used in drying rough rice, although temperatures above 100°F. (38°C.) have been reported (5) as adversely affecting the milling quality of rice. Since heat alone was not responsible for the breakage of the rice kernel, further studies on the effects of drying were made at Southern Utilization Research and Development Division of the U.S. Department of Agriculture, New Orleans, Louisiana. To determine the effects of drying temperatures on the cooking quality of the milled product, samples of rice which had been dried in unheated air and at elevated temperatures under conditions simulating commercial practice were evaluated by the Human Nutrition Research Division, Beltsville, Maryland.

The cooking qualities of two varieties of long-grain rice, Century Patna and Rexoro, were determined by panel evaluations of cooked rice, treatment of rice with water at 99°C., and treatment with dilute alkali.

Materials and Methods

Century Patna 231 and Rexoro varieties of rice were grown near Crowley, Louisiana, in 1954. Century Patna had been seeded April 8 and harvested August 31; Rexoro had been seeded April 25 and har-

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[2] Human Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

[3] Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, La.

vested October 31. Subsamples of each variety had been air-dried for 72 hours at temperatures from 70° to 82°F. (21°–28°C.), and dried in forced air by three passes through a model L.S.U.-type dryer (1) with temperatures of 120°F. (49°C.), 140°F. (60°C.), and 160°F. (71°C.). Operating variables for the forced-air dried samples are given in Table I.

Two hundred seventy-five pounds of each sample were milled in the pilot mill of the University of Arkansas at Stuttgart, Arkansas (2), at an approximate rate of 75 lb. of rough rice per hour. The procedure used in milling the samples was standardized as nearly as possible.

The milled samples were shipped to Beltsville, Maryland, in metal containers with slip-top lids and were stored at 40°F. (5°C.) in these cans until tested about 6 months later.

Panel Evaluation of Cooked Rice. To prepare rice for quality evaluation by a panel, 250 ml. of boiling distilled water were added to 100 g. of each rice sample, and the rice was cooked in a covered baking dish in an oven at 350°F. (176°C.). The cooking times, selected on the basis of preceding studies, were 28 minutes for Century Patna and 30 minutes for the Rexoro samples, after which samples were allowed to steam for 5 minutes uncovered in the oven before serving.

A panel of experienced tasters ranked the samples in order of decreasing quality of color, cohesiveness, and off-flavor, and they scored the samples on the same factors using a 9-point rating scale. In ranking, the four samples were arranged in order first for color, from most white to least white; next, for cohesiveness, from best separated grains to least separated; and, lastly, for absence or presence of off-flavor, from least to most off-flavor. Numbers from 4 to 1 were applied to each of the quality ranks. Scoring was done by the method described previously (4).

A randomized block design consisting of six sessions for each variety of rice was used for the panel tests. Three sessions were devoted to ranking and three to scoring of the samples. Four samples representing the four drying temperatures were presented at each session. Only one variety of rice was tested at a session, and all samples of Century Patna rice were tested before the Rexoro samples.

Treatment of Rice with Water at 99°C. Samples of rice were tested using equipment and procedures previously described (4). Measurements included water absorbed by a specified quantity of rice in a given length of time (designated as water-uptake ratio), volume of cooked rice, and total solids and estimated starch in the residual cooking liquid. Two samples of rice were cooked each day for 12, 16,

TABLE I

OPERATING VARIABLES FOR FORCED-AIR DRIED SAMPLES

VARIETY	DRYING TEMPERATURE		PASS[a]	WEIGHT ROUGH RICE	MOISTURE CONTENT[b]		TIME IN DRYER	TEMPERATURE OF RICE			
					Entering Dryer	Leaving Dryer		Entering Dryer		Leaving Dryer	
	°F.	°C.		lb	%	%	minutes	°F.	°C.	°F.	°C.
Century Patna 231	120	49	1	300	20.8	17.6	40	74	23	96	36
			2	290	17.6	14.9	30	83	28	105	41
			3	282	14.9	12.5	20	88	31	112	44
	140	60	1	300	21.3	17.7	34	78	26	93	34
			2	289	17.7	14.5	24	85	29	113	45
			3	280	14.5	12.8	15	89	32	117	47
	160	71	1	300	21.1	17.0	25	75	24	92	33
			2	288	17.0	13.6	25	83	28	117	47
			3	281	13.6	11.8	15	87	31	122	50
Rexoro	120	49	1	300	19.7	16.3	37	77	25	93	34
			2	290	16.3	13.4	30	81	27	104	40
			3	282	13.4	11.6	23	89	32	115	46
	140	60	1	300	19.9	16.8	30	75	24	98	37
			2	291	16.8	14.0	20	83	28	117	47
			3	283	14.0	12.1	20	87	31	119	48
	160	71	1	300	20.1	16.2	25	74	23	94	34
			2	288	16.2	13.2	21	82	28	115	46
			3	280	13.2	11.7	15	86	30	119	48

[a] For both varieties and all temperatures the air flow was 5300 c.f.m.; and the estimated surface air velocity 90 f.p.m. Samples were allowed to temper overnight in a closed 55-gal. drum between passes.

[b] Wet basis.

20, 24, and 28 minutes, and four replications were made of each measurement for each cooking time.

The following changes were made in the starch determinations: a) Residual cooking liquid was cooled for about 3 hours in a constant-temperature room at 75°F. (24°C.) prior to the determination in order to reduce variation among replications; b) readings were made on a Bausch & Lomb Spectronic 20 Spectrophotometer at 600 $m\mu$, since maximum absorption of dilute amylose-iodine solutions is at about this wave length.

Treatment of Rice with Dilute Alkali. Six replications of each sub-sample were immersed in a 1.7% solution of potassium hydroxide for 23 hours as described by Little *et al.* (6). Observations were made of the extent of kernel disintegration or spreading and subsequent clearing of opaque areas. Three replications were made in January and three in August 1956. Head rice and second heads were treated separately.

Results and Discussion

The results of the panel evaluations and measured effects of treatment with water and with dilute alkali are summarized in Tables II, III, and IV.

Statistically significant variations in some of the quality characteristics as determined by a panel, physical and chemical measurements, and response to alkali were observed for some of the rice samples dried at different temperatures. However, the variations were not linearly related to successive increases in temperature, nor were they similar for the two varieties of rice.

Panel Evaluations of Cooked Rice. Except for color, differences in the various quality characteristics of cooked Century Patna rice were not significant, whether ranking or scoring techniques were used (Table II). Century Patna rice had a slightly whiter color when dried at room temperatures or at 140°F. (60°C.) than at other temperatures.

Cooked samples of Rexoro rice dried at 120°F. (49°C.) appeared to have better color, less off-flavor, and less cohesiveness than samples of rice dried at other temperatures when evaluated by ranking. When scoring techniques were used for evaluating Rexoro rice, the differences in scores for the various quality characteristics were not significant.

The judges rated the samples of Rexoro rice in the drying experiment as slightly more cohesive or sticky and slightly poorer in flavor than other lots of this variety (3). The scores for the samples of Cen-

TABLE II

PANEL EVALUATION OF THE QUALITY OF COOKED RICE BY SCORING AND RANKING

VARIETY	DRYING TEMPERATURE OF ROUGH RICE		COLOR		COHESIVENESS		OFF-FLAVOR	
			Mean Score	Mean Rank	Mean Score	Mean Rank	Mean Score	Mean Rank
	°F	°C						
Century Patna	Room		8.2	2.5	6.1	2.4	8.1	2.3
	120	49	6.8	1.7	6.1	2.6	7.6	2.3
	140	60	7.6	3.0	5.7	1.9	8.1	2.3
	160	71	7.4	2.8	6.4	3.1	7.7	3.1
	TD[a]		0.8	1.2	n.s.	n.s.	n.s.	n.s.
Rexoro	Room		7.8	2.2	5.8	2.0	7.7	2.0
	120	49	8.0	3.3	6.2	3.2	7.9	3.5
	140	60	7.4	2.6	5.3	1.8	7.6	2.1
	160	71	7.4	1.9	6.5	2.9	8.1	2.4
	TD[a]		n.s.	1.0	n.s.	1.0	n.s.	1.0

[a] Differences greater than the test differences (TD) are significant at the 5% level. N.s. indicates no significant differences in the group of means to which it refers.

tury Patna in this study were within the range obtained for other lots of this variety.

Results of Treatment with Water at 99°C. Water-uptake ratio differed with drying temperature only for Century Patna variety; rice dried at 140°F. (60°C.) absorbed the most water. Differences in volume of cooked rice due to drying temperature were not significant for either variety of rice.

The residual cooking liquids for Century Patna rice dried at 140°F. (60°C.) had the most starch; that dried at 160°F. (71°C.), the least starch and total solids. The residual cooking liquids for Rexoro rice dried at 160°F. also had less starch than the liquids from rices dried at other temperatures; differences in total solids were not significant.

Both water-uptake ratio and starch content of the residual liquids for these samples were within the range obtained for corresponding lots previously tested (3). The total solids contents of the residual liquids for the samples of Rexoro rice were higher than those of previously tested lots of this variety.

Results of Treatment with Dilute Alkali. The mean values for spreading and for clearing observed during the August trials are shown in Table IV. In Century Patna 231 there was a little deviation from the usual behavior; that is, a few kernels developed a narrow, partial, powdery collar rather than remaining intact. However, the deviation was slight and seemed unrelated to drying temperature or milled size of the kernels.

The results from the treatment of the samples of rice with dilute

alkali were similar to results from other lots of the same varieties of rice previously reported (6).

TABLE III

TREATMENT OF MILLED RICE WITH WATER AT 99°C.: WATER-UPTAKE RATIO, VOLUME OF COOKED RICE, STARCH AND TOTAL SOLIDS IN RESIDUAL LIQUID

VARIETY	DRYING TEMPERATURE OF ROUGH RICE		COOKING TIME (MINUTES)				
			12	16	20	24	28
	°F	°C					
			Mean water-uptake ratio[a]				
Century Patna	Room		2.90	3.31	3.60	3.84	4.08
	120	49	2.93	3.31	3.61	3.88	4.18
	140	60	2.96	3.33	3.67	3.97	4.22
	160	71	2.89	3.32	3.60	3.86	4.07
	T.D.[b]				0.11		
Rexoro	Room		2.88	3.24	3.60	3.86	4.14
	120	49	2.92	3.18	3.48	3.72	3.98
	140	60	2.94	3.24	3.54	3.81	4.08
	160	71	2.92	3.19	3.52	3.81	4.09
	T.D.[b]				0.13		
			Mean volume of cooked rice (ml.)[c]				
Century Patna	Room		41	46	52	55	57
	120	49	41	47	50	56	56
	140	60	41	46	50	54	56
	160	71	39	46	49	53	56
Rexoro	Room		38	45	49	54	56
	120	49	40	44	50	52	55
	140	60	40	47	50	54	56
	160	71	38	44	49	52	54
			Mean values for starch in residual liquid (g.)				
Century Patna	Room		0.34	0.48	0.62	0.72	0.83
	120	49	0.31	0.43	0.49	0.64	0.77
	140	60	0.36	0.51	0.70	0.76	0.90
	160	71	0.31	0.42	0.50	0.61	0.69
	T.D.[b]				0.10		
Rexoro	Room		0.75	1.02	1.22	1.60	1.78
	120	49	0.79	1.01	1.28	1.55	1.81
	140	60	0.81	1.06	1.31	1.61	1.82
	160	71	0.70	0.95	1.18	1.43	1.61
	T.D.[b]				0.18		
			Mean values for total solids in residual liquid (g.)				
Century Patna	Room		0.53	0.73	0.92	1.02	1.12
	120	49	0.53	0.74	0.81	0.95	1.15
	140	60	0.56	0.71	0.92	1.09	1.19
	160	71	0.51	0.62	0.75	0.92	1.01
	T.D.[b]				0.11		
Rexoro	Room		0.59	0.80	0.95	1.20	1.31
	120	49	0.60	0.80	1.00	1.20	1.36
	140	60	0.64	0.84	1.04	1.23	1.31
	160	71	0.60	0.77	1.02	1.22	1.30
	T.D.[b]				0.16		

[a] Weight of cooked rice divided by weight of uncooked rice.
[b] T.D.: Differences greater than the test difference are significant at the 1% level.
[c] Mean volume of cooked rice from 8 g. uncooked rice (ml.).

TABLE IV

VALUES FOR SPREADING AND CLEARING OF RICE TREATED WITH DILUTE ALKALI

VARIETY	DRYING TEMPERATURE OF ROUGH RICE		SPREADING[a]		CLEARING[b]	
			Head Rice	Second Heads	Head Rice	Second Heads
	°F	°C				
Century Patna	Room		2.1	2.1	1.1	1.1
	120	49	2.0	2.1	1.0	1.1
	140	60	2.1	2.0	1.1	1.0
	160	71	2.0	2.0	1.0	1.0
Rexoro	Room		4.0	4.0	3.3	3.1
	120	49	4.0	4.0	2.8	2.8
	140	60	4.0	4.0	2.9	2.8
	160	71	3.9	3.8	3.4	2.8

[a] Spreading scale: 1, kernel not affected; 2, kernel swollen; 3, kernel swollen, collar incomplete or narrow; 4, kernel swollen, collar complete and wide; 5, kernel split or segmented, collar complete and wide; 6, kernel dispersed, merging with collar; 7, all kernels completely dispersed and intermingled.

[b] Clearing scale: 1, kernel chalky; 2, kernel chalky, collar powdery; 3, kernel chalky, collar cottony or cloudy; 4, center cottony, collar cloudy; 5, center cottony, collar clearing; 6, center cloudy, collar cleared; 7, center and collar cleared.

Acknowledgment

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COMPARATIVE STUDY OF WHEAT FLOUR PHOSPHATIDES[1]

L. H. Mason and A. E. Johnston

ABSTRACT

Lipids from two hard red winter wheat flours, Ponca and Red Chief, differing in bread-baking quality were extracted with water-saturated butanol-1 and fractionated with solvents to study composition differences. Phosphatide fractions from each of the flours were subjected to 800 transfer countercurrent distributions, using the solvent system water:methanol:water-saturated butanol:1-heptane (3:17:40:60). In a large number of the fractions in both distributions, the molar nitrogen-to-phosphorus ratio was very nearly 1. Weight curves in general were similar; each showed a major peak comprised of nearly pure digalactosyl glycerides. A major quantitative difference was found in another large peak of both curves which contained a large proportion of choline phosphatides with more material in the distribution from Red Chief, the flour of poor baking quality.

Wheat flour lipids have been shown to have a considerable influence on the bread-baking quality of flour (10,13,22). Coppock *et al.* (13) found that the acetone-insoluble fraction of wheat flour lipids largely restored the baking quality of flour defatted with carbon tetrachloride-ether. Shortening, glycerinated fats, and lecithin had either no effects or only small adverse ones. In contrast to earlier reports (11,13), recent work has indicated that the acetone-insoluble fraction restored only a little of the baking quality to defatted flour (12). The explanation given for the discrepancy in results was based on differences in the flours used. Flours examined more recently were of lower extraction and, more important, were from wheats grown in cool damp weather.

Cookson *et al.* (12) fractionated flour lipids using two 23-transfer countercurrent distributions with the solvent systems heptane-methanol and carbon tetrachloride-methanol-water. The contents of each distribution were combined into four fractions. Bread-baking tests of the eight fractions showed that separations had been achieved. Oily fractions had adverse effects and waxy fractions improved the baking quality of defatted flour.

To investigate the composition of wheat flour lipids and the relation of composition to baking performance, lipids of two flours, comparable except for baking quality, were studied using both solvent fractionation and countercurrent distribution.

Materials and Methods

Milling. Two hard red winter wheats were used to obtain the flours studied—Ponca which yields good bread flour and Red Chief which

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yields poor bread flour. These wheats were cleaned with a scourer, tempered to 14% moisture, and held for 2 days. The moisture content was raised from 14 to 15% in 1 day and to 15.5% half a day later, just prior to milling with a Buhler[2] mill. The flours were stored at 0°C. and used within 2 weeks. Milling and composition data are presented in Table I.

TABLE I
FLOUR MILLING AND COMPOSITION DATA

	PONCA	RED CHIEF
	% of wheat	*% of wheat*
Flour	68.3	63.5
Bran	18.3	20.5
Shorts	10.8	13.0
Loss	2.5	3.0
	% of flour	*% of flour*
Flour ash[a]	0.037	0.037
Flour protein[a]	12.8	10.9

[a] These data are corrected to 14% moisture.

Extraction. Test extractions were made using ethanol-ether and water-saturated butanol. Two-pound batches of flour were boiled 30 minutes with 1,600 ml. of 95% ethanol, filtered, and washed on the filter twice with 500-ml. portions of peroxide-free ethyl ether. This extraction process was repeated once. The combined ethanol and ether extracts were concentrated to dryness *in vacuo*.

For the water-saturated butanol test extractions, a Waring Blendor was used with 2-lb. batches of flour, and a 5-gal. pail with air-driven propeller mixers was used with 5-lb. batches. In both cases a solvent-to-solid ratio of 2 ml. of water-saturated, redistilled commercial butanol-1 per g. of flour was used. Two 1-hour extractions were made at room temperature using filtration to separate the flour from the extract. The combined butanol extracts were concentrated to dryness *in vacuo*. When dried *in vacuo* the final flour cake released last traces of butanol with difficulty and became very hard. In contrast, a fine white powder was obtained when the flour cake from the ethanol-ether extraction was air-dried.

Preparative-scale extractions were made using a 50-gal. kettle equipped with a paddle stirrer and baffle. Filtered commercial butanol-1 was saturated with distilled water in the kettle. After excess water was removed, the flour (82 lb. of Ponca or 127 lb. of Red Chief) was added slowly with the stirrer in operation. The final ratio of solvent-

[2] Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture.

to-flour (2 ml. per g.) yielded a thick slurry which was stirred 1 hour at room temperature and was easily filtered with suction. The cake was then dispersed into a second batch of wet butanol and the slurry stirred for another hour. Filtration of this slurry was considerably more difficult because the flour had become doughy. Dry butanol does not cause flour to become doughy, but it is not as effective as wet butanol for extraction of lipids. Concentration *in vacuo* of the combined extracts yielded a viscous golden-brown syrup.

Solvent Fractionation. Fractionation of the extract from 127 lb. of Red Chief flour is described as typical. Volumes of solvents used to fractionate extracts from other amounts of flour were in similar proportions.

The dried butanol extract (680 g.) was dissolved in 6 liters of USP chloroform and filtered through a pad of glass wool to remove a small amount of white particulate material. The chloroform was removed by concentration *in vacuo* and the residue (chloroform-soluble material) was dissolved in a mixture of 600 ml. of redistilled pentane-hexane and 100 ml. of chloroform in preparation for an acetone precipitation. This solution was concentrated *in vacuo* to a thin slurry. Six liters of redistilled acetone were added slowly with vigorous stirring. After storage of the resulting mixture overnight at 5°C., the supernatant was decanted through a glass-wool pad. Precipitates on the wool and in the flask were redissolved in pentane-hexane and chloroform as described above and were twice more precipitated, stored overnight, and decanted. For the second overnight storage, temperature was 5°C. and for the third, 25°C. After the last decantation, the combined acetone filtrates were concentrated and dried *in vacuo*.

The light-tan acetone-insoluble material was dried *in vacuo* to remove acetone and dissolved in 3 liters of pentane-hexane. This solution was washed twice with 50% aqueous ethanol according to Scholfield *et al.* (25). During the second wash an emulsion formed which had to be broken by centrifugation. The combined aqueous ethanol washes were shaken with 1,500 ml. of pentane-hexane and after removal of the pentane-hexane were concentrated *in vacuo* using butanol as an antifoam. Concentration of the pentane-hexane layers yielded the desired phosphatides. However, when the acetone-insoluble lipids from Ponca flour were given the second aqueous ethanol wash, the emulsion could not be broken by centrifugation for 1 hour. Addition of 6% of butanol-1 caused the emulsion to break rapidly. The first alcohol wash was shaken with a half-volume portion of pentane-hexane and the second wash with two half-volume portions. Alcohol washes and

pentane-hexane layers were concentrated as described.

Countercurrent Distribution. A 200-tube automatic countercurrent distribution apparatus was used with 40 ml. of each phase per tube and a co-current flow of 1 ml. of lower phase per transfer in order to maintain volume. The solvent system consisted of 6 liters of water-saturated redistilled commercial butanol-1, 9 liters of redistilled commercial *n*-heptane, 2,550 ml. reagent grade methanol, and 450 ml. distilled water,[3] thoroughly mixed together with an air stirrer.

Eight grams of phosphatide were dissolved in a total of 800 ml. of upper-plus-lower phase, such that the phases were of equal volume after mixing with solute and settling. Tubes 0 to 9 were filled with 40 ml. of each layer of the phosphatide solution. All other tubes were provided with 40 ml. of the lower phase solvent. After the first ten transfers the settling time had to be increased to 40 minutes because of emulsion formation. At later stages in the distributions the emulsions were not completely broken after 40 minutes but were confined to the bottom phase and were, therefore, not transferred. Three weeks were required for each 800-transfer distribution of phosphatides. As the upper layers emerged from tube 200 after transfer 190, they were collected to form the withdrawn series, according to the single withdrawal method of Craig and Craig (14). On the basis of a curve made by weighing the residues from every fifth tube, the tube contents were combined for analytical study.

Analytical Methods. Nitrogen was determined by a standard micro-Kjeldahl method, phosphorus by the method of Bernhart and Wreath (5), galactose essentially by the anthrone method of Radin *et al.* (24), choline as the reineckate according to Glick (17), long chain base nitrogen by the method of Carter *et al.* (8), and the sum of serine and ethanolamine by the method of Burmaster (7). Samples for inositol determinations were refluxed 24 hours with 20% hydrochloric acid. Excess acid was removed by evaporation and passage over Amberlite IR-4 resin. Inositol was then determined by a modification of the pyridoxine assay method of Atkin *et al.* (3).

For fatty acid, nonsaponifiable material, and nonionic sugar determinations, 3-g. samples were refluxed 2 hours with 120-ml. portions of 0.5*N* aqueous potassium hydroxide. After cooling, the solutions were acidified with sulfuric acid and extracted with ether. The ether layers from each sample were washed with two small portions of water. Amberlite MB-3 monobed resin was used to deionize each combined water layer and washes. Concentration of the deionized solution yield-

[3] Personal communication from H. E. Carter and D. S. Galanos, University of Illinois, Urbana, Ill.

ed nonionic sugar fractions containing glycerol, monosaccharides, and galactosyl glycerols.

The ether extracts containing the fatty acids and nonsaponifiable material were washed twice with 50-ml. portions of 0.7*N* aqueous potassium hydroxide and four times with 50-ml. portions of water. These ether layers were dried over sodium sulfate and evaporated to yield nonsaponifiable material.

To isolate fatty acids the alkaline washes were acidified with sulfuric acid and extracted three times with *n*-heptane. Removal of sulfuric acid from the heptane solution was effected by washing three times with small volumes of half-saturated sodium sulfate. Anhydrous sodium sulfate was used to dry the heptane solutions before these were concentrated *in vacuo* to yield fatty acids.

Iodine values were determined by a microadaptation of the AOCS official method Cd 1-25 (1), using 25-mg. samples.

Neutral equivalents were obtained by titration of 5-mg. samples in benzene solution with 0.246*N* alcoholic potassium hydroxide using 0.003% thymol blue in 95% alcohol as the indicator.

Fatty acids were esterified with diazomethane according to Arndt (2).

Isolation and purification of phytoglycolipid were performed according to Law (19).

Paper Chromatography. Silicic acid-glass fiber paper was prepared according to Dieckert and Reiser (15), eliminating the methanol and ether washes. Solvent systems used for phosphatides were 20% methanol in chloroform, and 95% ethanol (20).

Whatman No. 1 paper was used for amino acid and sugar paper chromatography with 75% phenol in water and butanol-pyridine-water (6:4:3) as solvents.

Staining procedures were as follows: For choline and choline phosphatides the paper was wet with 2% aqueous phosphomolybdic acid, rinsed with water, and soaked in water for 5 to 10 minutes. The blue spots on white background were developed by immersing the washed papers in 0.4% stannous chloride in 4*N* hydrochloric acid. To visualize amino acids the papers were sprayed with 0.25% ninhydrin in 50% aqueous pyridine, dried in a hood, and heated at 110°C. for 5 minutes. For carbohydrates the papers were sprayed with ammoniacal silver nitrate (five parts 0.1*N* aqueous silver nitrate plus one part 5*N* ammonia). The air-dried papers were heated at 110°C. until spots developed (monosaccharides about 5 minutes; glycerol and galactosyl glycerols about 10 to 15 minutes). The periodic acid-potassium iodide spray method of Metzenberg and Mitchell (23) and an aniline acid phthalate

spray (6) were also used to visualize carbohydrates.

To study the nitrogen compounds in the phosphatides, 20-mg. samples were refluxed 6 hours with 10 ml. of 6*N* hydrochloric acid and the acid removed in an evacuated desiccator over potassium hydroxide pellets. After addition of water, the samples were redried, redissolved in 2 ml. of water, and measured amounts applied to the papers. The pH's of the solutions were about 4.

Paper chromatograms of fatty acids were run essentially by the method of Gellerman and Schlenk (16) using soluble starch in place of alpha-dextrin and DC 550 oil in place of DC 200 oil.

Gas Chromatography. Celite 545, an inert support for gas chromatograph columns, was prepared and coated with stationary phase in a manner similar to that of James and Martin (18). Celite was size-graded using a Ro-Tap shaker with standard sieves, washed with hydrochloric acid, water, methanol, 5% methanolic potassium hydroxide, and methanol in that order, and dried overnight at 160°C. Stationary phases (2.5 g. per 10 g. of Celite) were dissolved in 100 ml. of pentane-hexane. Washed Celite was added to this solution which was stirred vigorously and heated on a steam bath to remove pentane-hexane. Final drying was done overnight at 120°C. Stainless-steel tubes ¼ in. o.d., 2 to 5 ft. long, were plugged at one end with Pyrex glass wool and packed with stationary phase-on-Celite using vibration supplied by holding the tubes against a rotating, flattened motor shaft. Vibration was stopped when no more compacting was observed. The open end was plugged with glass wool and the tube formed into a 6-in. coil to fit an Aerograph gas chromatograph.

Gas-chromatographic analyses of methyl esters of flour lipid fatty acids were performed, using a 5-ft. column of Apiezon L grease on silane-coated Celite 545 (60–80 mesh) at 242°C. in the Aerograph. Filament current was 200 ma. with a sensitivity of 10 mv. Helium, the carrier gas, was used at a flow rate of 29 ml. per minute (S T P). Sample size was 5 μl. Methyl esters of digalactosyl glyceride fatty acids were analyzed at 220°C. in the Aerograph equipped with a commercial 5-ft. silicone column. A flow rate of 90 ml. per minute (S T P) of helium was employed. Filament current and sensitivity were 200 ma. and 10 mv., respectively.

Isolation of Digalactosyl Glycerol. One-gram samples from tubes 171–199 in the fundamental series of the Red Chief distribution and from tubes 176–199 in the fundamental series of the Ponca distribution were refluxed 2 hours with 15 ml. of 0.5*N* aqueous potassium hydroxide. After acidification the fatty acids were extracted with ether. The

aqueous solutions were deionized with Amberlite MB-3 monobed resin, concentrated, and freeze-dried. Hot methanol solutions of the dried, fluffy white solids were prepared. On cooling to 25°C. the solutions deposited coarse rosettes. Recrystallization from aqueous ethanol yielded rosettes of needles.

Periodic acid oxidation studies were performed according to Carter *et al.* (9).

Results and Discussion

Isolation of the Phosphatides. Test extractions of a mixture of 67% hard winter wheat flour and 33% hard spring wheat flour were made to compare the ethanol-ether method used in unpublished work from this laboratory with the water-saturated butanol method adapted from Mecham and Mohammad (21).

Results of these tests, shown in Table II, indicate that water-saturated butanol is more effective in removing lipids, particularly phosphatides from wheat flour. The ethanol-ether method removed a large amount of chloroform-insoluble, flourlike material which increased the yield of that extract. Since water-saturated butanol extracted more lipids and since it can be used at room temperature, it was selected for preparative-scale extractions of the Red Chief and Ponca flours.

Yields of extracts and of fractions derived from them, as compared in Table III, indicate more phosphatides in the poor Red Chief flour and more ethanol wash material in the good Ponca flour. The small amount of chloroform-insoluble material present in both extracts had the appearance of flour and had the following percentage composition: sulfur, 0.17; nitrogen, 1.95; phosphorus, 0.05; anthrone carbohydrate, 97.4.

During the 50% ethanol-wash procedure, the purpose of which is

TABLE II
COMPARATIVE TEST EXTRACTIONS OF HARD WHEAT FLOUR

	Yields	
	Alcohol-Ether	Water-Saturated Butanol
	% of flour	*% of flour*
Extract	3.11	1.44
Chloroform-soluble	1.15	1.20
Acetone-soluble	0.78	0.73
Acetone-insoluble	0.27	0.38
50% Ethanol wash	0.06	0.10
Phosphatides	0.22	0.28

to remove nonlipid carbohydrate, the Ponca acetone-insoluble lipids formed stable emulsions more readily than did the acetone-insoluble lipids of Red Chief, the poorer flour.

Phosphatide Composition. Close similarities in composition between phosphatides and between acetone-soluble fractions of the two flours are shown by data in Table IV. However, there are marked differences in the content of phosphorus, galactose, and fatty acids of the ethanol-wash fractions from the two flours.

Since data in Table IV indicate the presence of only small amounts of inositol, it is concluded that wheat endosperm contains very little inositol lipids. Wheat endosperm also contains very little phytoglycolipid – a lipid containing inositol, phosphate, glucosamine, hexuronic acid, arabinose, mannose, galactose, and lignoceric and cerebronic amides of phytosphingosine (19). Phytoglycolipid has been isolated from corn, soybean, flaxseed, and wheat germ (19). Mild alkaline hydrolysis of Ponca and Red Chief lipid fractions followed by pyridine purification (19) of the precipitates revealed the presence of very little

TABLE III
SOLVENT FRACTIONATION OF FLOUR LIPIDS

	Yields	
	Ponca	Red Chief
	% of flour	*% of flour*
Butanol extract	1.16	1.18
Chloroform-insoluble	0.006	0.005
Chloroform-soluble	1.16	1.17
Acetone-soluble	0.59	0.57
50% Ethanol wash	0.24	0.11
Phosphatides	0.29	0.49

TABLE IV
ANALYSIS OF FLOUR LIPID FRACTIONS

	Percentage Values						
	Nitrogen	Phosphorus	Galactose	Fatty Acid	Non-Saponifiable	Inositol	Ash
RED CHIEF							
Acetone-soluble	0.18	0.11	6.6	58.7	8.20	0.015	0.25
Alcohol wash	2.00	0.69	85.5	5.6	0.72	0.28	4.86
Phosphatides	1.03	1.94	18.3	44.4	7.84	0.073	3.18
PONCA							
Acetone-soluble	0.27	0.10	5.8	66.0	5.60	0.005	0.21
Alcohol wash	2.28	1.29	57.0	19.3	2.85	0.13	5.17
Phosphatides	0.90	1.71	19.8	46.4	6.54	0.067	3.10

or no phytoglycolipid in the acetone-soluble and alcohol-wash fractions; there was 0.5% and 0.6% in Ponca and Red Chief phosphatides, compared with 4% isolated from soybean phosphatides.

Analyses for individual nitrogen-containing compounds are presented in Table V. Paper chromatograms indicate that the large amounts of unknown nitrogen in the alcohol washes represented free

TABLE V

NITROGEN DISTRIBUTION IN THE FLOUR LIPID FRACTIONS

	Nitrogen			
	Choline	Serine and Ethanolamine	Long Chain Base	Unknown
	%	%	%	%
Red Chief				
Acetone-soluble	0.00	0.04	0.05	0.09
Alcohol wash	0.25	0.04	0.02	1.69
Phosphatides	0.49	0.28	0.09	0.17
Ponca				
Acetone-soluble	0.00	0.03	0.01	0.23
Alcohol wash	0.40	0.13	0.05	1.70
Phosphatides	0.34	0.28	0.19	0.09

and combined amino acids. The relatively large amounts of choline present in the alcohol washes is probably in the form of glycerylphosphoryl choline or lysophosphalidyl choline.

Gas-liquid partition chromatography was used to study the composition of fatty acid methyl esters from the flour lipid fractions. It was not possible to differentiate amounts of methyl stearate less than 10% of the total from the esters of the unsaturated 18-carbon acids. Chromatograph results and iodine values, shown in Table VI, indicate a general similarity, although the acetone-soluble fractions are more highly unsaturated and contain less palmitic acid.

Paper chromatographic studies of the nonionic sugar fractions showed that most of the carbohydrate in the phosphatides was digalactosyl glycerol (α-D-galactopyranosyl-1,6-β-D-galactopyranosyl-1-glycerol (9), the identification of which is described later in this paper). Very little monogalactosyl glycerol (β-D-galactopyranosyl-1-glycerol (9)) was found in any of the lipid fractions, and only traces of glucose and galactose were found. No evidence of pentoses was seen.

Countercurrent Distribution. Weight and analytical curves of Ponca and Red Chief phosphatide countercurrent distributions shown in Figs. 1 and 2 are generally similar except for peaks in the regions of tubes F (fundamental) 60 to F110. In Fig. 1 the weight curve in this

TABLE VI

ANALYSES OF FLOUR LIPID FATTY ACIDS

	Iodine Value	C_{18}	C_{18}
		%	%
Red Chief			
Acetone-soluble	143	15.5	84.5
Alcohol wash	125	33.0	67.0
Phosphatides	132	25.0	75.0
Ponca			
Acetone-soluble	146	16.7	83.3
Alcohol wash	117	21.2	78.8
Phosphatides	125	24.2	75.8

region has a shape which closely approaches that of a theoretical distribution curve. Such a shape should indicate homogeneity; however, this same region in Fig. 2 is composed of several peaks. These peaks, as well as the rest of the distribution fractions, were investigated, using the silicic acid-paper chromatographic technique of Lea *et al.* (20). Results, shown in Table VII, indicate that neither of the F60 to F110 regions is homogeneous. In this region the Red Chief peak contains lyophosphatidyl ethanolamine which is absent in the Ponca peak. Both peaks contain lyophosphatidyl choline and phosphatidyl choline, with the Red Chief peak including more total material.

In the withdrawn (W) series of both distributions, phosphatidyl

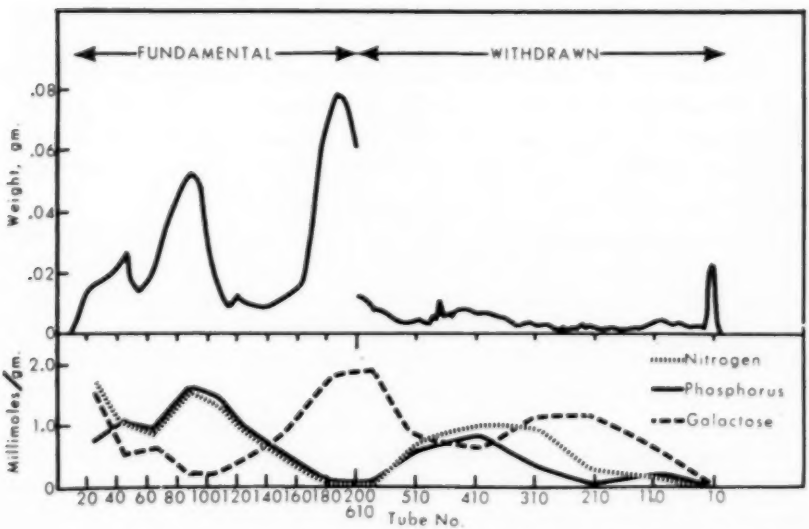

Fig. 1. Countercurrent distribution of Red Chief flour phosphatides.

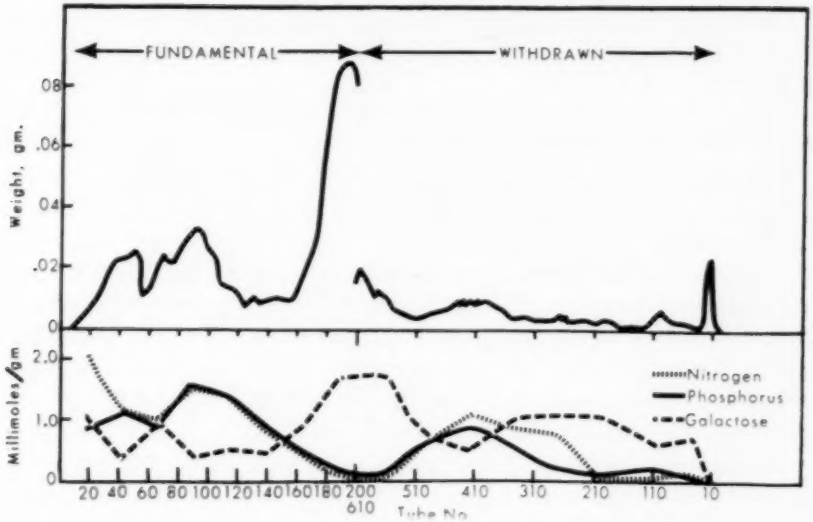

Fig. 2. Countercurrent distribution of Ponca flour phosphatides.

TABLE VII

COUNTERCURRENT DISTRIBUTION OF WHEAT FLOUR PHOSPHATIDES[a]

Red Chief Phosphatides Distribution Tube No.	LPC	LPE	PC	PE	PS	Ponca Phosphatides Dis. tribution Tube No.	LPC	PC	PE	PS
11–30						6–30				
31–55	+			++	+	31–55			++	+
56–75	++			++	+	56–75	+		++	
76–100	++	+++	+++	+		76–100	+	+++	+	
101–115	+++	++	+	+		101–125	+	+++	+++	
116–135	++			++	+++	126–155			+++	+
136–170				+	+++	156–175			++	+
171–200				+		176–200			+	
551–610			+	+	+	551–610			+	
472–550			++++	++	+	471–550		+++		
336–471			++++	+	+	371–470		++++	+	+
275–335			+++			301–370		+++	++	
151–275				+	+	246–300		+	+	+++
31–150					+	146–245			+	+
0–30						71–145				+
						0–70				

[a] LPC = Lysophosphatidyl choline; LPE = lysophosphatidyl ethanolamine; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PS = phosphatidyl serine. The plus signs indicate the relative amounts of material.

choline is concentrated in the area of tubes W300 to W550 with more total material in the Ponca distribution. Phosphatidyl ethanolamine and phosphatidyl serine are spread throughout both distributions with only a few indications of concentration.

McKibben-Taylor analyses indicated that long chain bases were concentrated in tubes F31 to F55 and W300 to W470 of both distributions.

Nonpolar, heptane-soluble material is contained in the peak in tubes W0 to W30 in each distribution.

Although large amounts of phosphatidic acids have been reported (4) to occur in flour lipids, in the present work the amounts are limited to 20% by the ash values in Table IV and to much less than 10% by the nitrogen and phosphorus analytical curves in Figs. 1 and 2. Presence of phosphatidic acids requires molar nitrogen-to-phosphorus ratios less than 1, unless compounds are present with nitrogen-to-phosphorus ratios greater than 1. It appears unlikely that there are large amounts of compounds with nitrogen-phosphorus ratios greater than 1, but this point needs to be investigated.

Galactosyl Glycerides. Analytical data in Figs. 1 and 2 indicate the presence in the tall narrow peaks (F170 to F200) of material containing about 30% galactose and very little nitrogen or phosphorus. Work reported by Carter *et al.* (9) indicated that this material should be glycerides of mono- and digalactosyl glycerol. From material in these peaks of both distributions digalastosyl glycerol was readily isolated in good yield. A comparison is made in Table VIII of properties of digalactosyl glycerol isolated from Ponca and Red Chief distribution peaks with digalactosyl glycerol isolated from lipids of bleached wheat flour by Carter *et al.* (9). Infrared spectra and paper-chromatographic behavior of all three digalactosyl glycerol samples were identical.

Only traces of monogalactosyl glycerol were found, in contrast to the findings of Carter *et al.* (9), who report that as much as 50% of one of their galactosyl glycerol fractions was monogalactosyl glycerol. It may be that bleaching caused hydrolytic removal of one galactose molecule from digalactosyl glycerol, or that the soft wheats studied by Carter have compositions different from the hard wheats used in the present study.

Fatty acids of digalactosyl glycerides were analyzed as the methyl esters, using the Aerograph gas chromatograph. It was found that 15% of the acids were C_{16} in length and 85% were C_{18} with no evidence visible for shorter chain acids.

Flour lipid galactosyl glyceride content should be considered in any determination of monoglycerides using periodic acid (13), because galactosyl glycerides consume four times as much periodic acid and liberate the same amounts of formaldehyde as do monoglycerides.

Calculations based on analytical data in Table IV show that diga-

TABLE VIII

COMPARISON OF DIGALACTOSYL GLYCEROLS

PROPERTIES	PONCA	RED CHIEF	CARTER[a]
Percentage of glyceride	38	42	....
Melting point °C.	194–196	194–196	194–196[b]
Analyses: Carbon	43.37[c]	43.36	....
Hydrogen	6.81	6.82	....
Optical rotation in water	+90.7°[d]	+89.4°[d]	+86.4°
	Periodic acid oxidation (moles per mole)		
Periodic acid reduced	5.01	5.09	5.0
Formic acid produced	2.07	2.05	1.9

[a] See reference 9.
[b] Melting point redetermined on a sample supplied by Dr. Carter. Lit. value is 182–184°C. (9). Discrepancy has not been explained.
[c] Calculated for $C_{15}H_{28}O_{14}$: C, 43.26; H, 6.78.
[d] Concentration = 3%.

lactosyl glycerides make up as much as 40% of the phosphatides. This percentage indicates the importance of these glycerides to bread quality. Their structure suggests that they should be good bread softeners. However, the small (1.5%) difference between galactose content of the phosphatides of the two flours indicates that digalactosyl glycerides are not wholly responsible for differences in flour baking quality. Instead, this observation plus the work of Coppock *et al.* (which indicated that lecithin had little effect) suggests that phosphatides of ethanolamine might be responsible for differences in bread-baking quality. In addition, it may be that emulsion characteristics are likewise related to baking quality through these phosphatides. Apparently more phosphatidyl ethanolamine is present in Ponca, the good flour, than in Red Chief, the poor flour.

Acknowledgment

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CONTINUOUS BATTER PROCESS FOR SEPARATING GLUTEN FROM WHEAT FLOUR[1]

R. A. Anderson, V. F. Pfeifer, and E. B. Lancaster

ABSTRACT

A continuous pilot plant for carrying out the batter process for the separation of starch and gluten from wheat flour has been designed and constructed. Flours milled from different types of wheat have been successfully processed in this plant. In most cases, the recovery of protein in the gluten has been greater than 80%, with the gluten purity also about 80%. The pilot plant, which is made up of conventional equipment, is quite versatile and amenable to simple scale-up.

Several methods are described in the literature for producing starch and gluten from wheat. Formerly whole-wheat kernels were processed, but more recently wheat flour has been used as the starting material to make wheat starch. One of the older methods employing flour is the so-called Martin, or dough-ball process, which involves washing starch from a stiff dough made out of flour and the proper quantity of water. Gluten produced by the Martin process is undenatured and, if dried carefully, yields a product of good quality (5). Modifications of this process are in use today in Europe and in two wheat-starch factories in the United States.

During World War II, workers at the Northern Utilization Research and Development Division developed two laboratory processes for separating starch and gluten from wheat flour. The first, called the alkali process, is chemical in nature and produces a denatured protein, which cannot be utilized in the same manner that "native" or undenatured gluten is used (4).

The second process, called the "batter process," involves mixing flour and water in proper proportions to give an elastic, but free-flowing, smooth batter (6,7). After this mixing operation, the batter is broken up mechanically in the presence of additional cold water, and the starch is quickly and almost completely washed out, leaving the gluten suspended in the slurry in the form of lumps or curds. The gluten is separated from the starch by screening the slurry on a gyrating shaker. The starch milk is tabled or centrifuged to yield a prime starch having a protein content of about 0.3%. The crude gluten passing over the end of the screen contains 60 to 65% protein. With one additional washing the protein content of the gluten is raised to about

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75–80%. This gluten is undenatured and, if dried carefully, maintains its vital characteristics.

The batter process was used commercially during World War II and for some time thereafter, when there was a shortage of corn on the cash market and an increasing demand for starch and its conversion products. However, its industrial application was a scale-up of the laboratory procedure, since time was not available then to carry out any developmental studies. According to the patent literature, some modifications were made to improve the batter process by commercial concerns adopting it. When the supply of corn became more plentiful and wheat was no longer considered an economical source of starch, commercial use of the batter process disappeared.

Because of recent interest in wheat as an industrial raw material and because of increasing recognition of wheat gluten as a source for protein, the Northern Utilization Research and Development Division initiated engineering studies directed toward the development of a more economical and efficient method of carrying out its batter process, preferably in a continuous fashion. This paper describes a continuous pilot plant and discusses its application in processing a number of flours milled from different types of wheat.

Description of the Continuous Pilot Plant

Figure 1 is a flowsheet for the continuous pilot plant for separating starch and gluten from wheat flour by the "batter process." The various

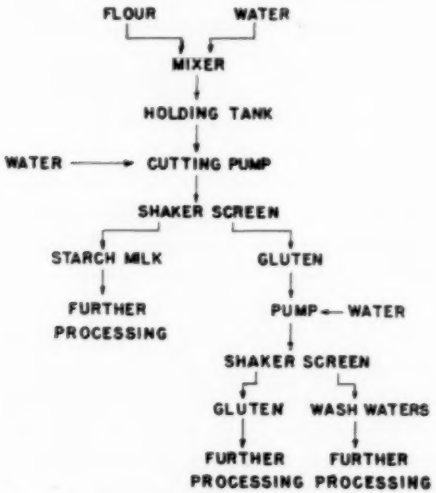

Fig. 1. Flow sheet of continuous pilot plant for fractionating wheat flour. Further processing of the fractions can be done by well-known procedures. Methods of purification depend on the end use of the particular products.

pieces of equipment which make up the pilot plant are simple, conventional, and readily available.

Wheat flour and water are fed continuously at controlled rates to the feed end of an Abbé double spiral ribbon blender,[2] in which the outer spiral blade carries material to the discharge end of the mixer while the inner blade works it back. The ratio of water to flour ranges between 0.7 to 1 and 1.8 to 1, depending upon the type of flour used. For a flour milled from a soft wheat, the ratio of water to flour ranges between 0.7 to 1 and 1.2 to 1; whereas with flours from hard wheats, the ratio ranges from 1.2 to 1 to 1.6 to 1. Flours of very high protein content may require a ratio of water to flour as high as 1.8 to 1. Water at a temperature of 120°–135°F. (48–57°C.) is used to facilitate hydration of the gluten and to reduce the mixing time necessary for producing a batter of proper consistency. The temperature of the batter during mixing is usually about 110°F. (43°C.). A view of the batter mixing in the blender is seen in Fig. 2.

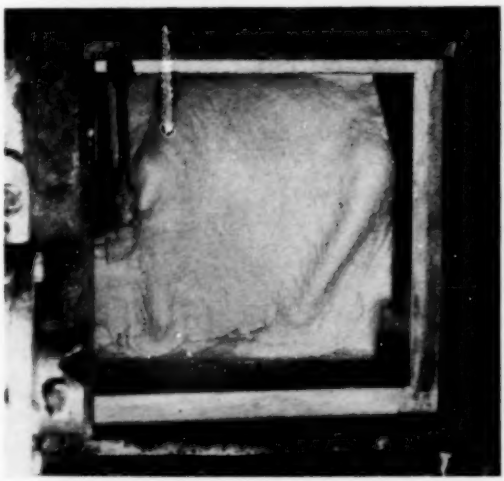

Fig. 2. Continuous mixing of flour and water to form a smooth batter in the double spiral ribbon blender.

After a short retention time in the mixer, the batter is in suitable condition for washing. If the flour has been milled from soft wheat, a longer retention time may be required which can be provided in the mixer itself, or in a separate agitated tank for additional holding or aging. The mixer and the holding tank are shown in Fig. 3.

The batter is then passed to a Jabsco pump, called the "cutting pump," together with sufficient cold water to give a ratio of water to

[2] The mention in this article of firm names or commercial products under proprietary names or names of their manufacturers does not constitute an endorsement by the U. S. Department of Agriculture of such firms or products.

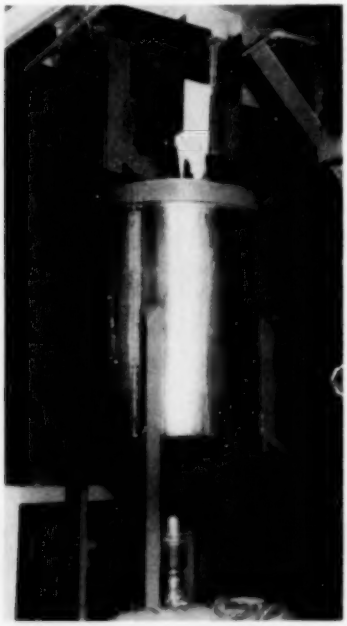

Fig. 3. Mixed batter discharging from overflow dam of the continuous mixer and dropping to the holding tank.

flour in the mixture at this point ranging from 2 to 1 to 5 to 1. Here the batter is intimately contacted with wash water by agitation of the pump impeller. Thus, starch is washed from the gluten, which remains in the form of small curds.

Slurry from the cutting pump is screened on a Rotex shaker, shown in Fig. 4. The curds of gluten are separated continuously from the starch milk on a screen of 60–150 mesh, depending upon the flour being processed. Wash water is sprayed on the separated gluten as it moves down the screen. Gluten recovered in this primary separation stage may have a protein content as high as 65%, dry basis.

The screened gluten is washed by pumping it, with added water, to a second shaker screen, shown in Fig. 5. Protein content of the gluten recovered after the second washing stage is in the range of 75–80%, dry basis. Extra washings can be used to increase further the protein content of the gluten.

Variables involved in the continuous process, such as water-to-flour ratio and retention time, are estimated by measuring changes in the mixing characteristics of small samples of batter prepared from the same flour by means of a sensitive torque measuring device described by Anderson and Lancaster (2).

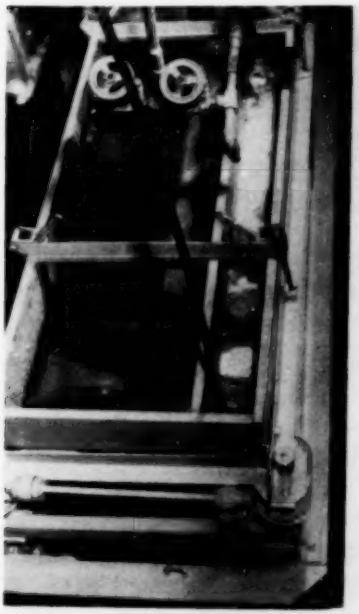

Fig. 4. Crude gluten separating continuously from starch milk on the first shaker screen, after dilution of the smooth batter.

Fig. 5. Checking strength of the wet gluten after washing and separating from wash waters on the second shaker screen.

Each of the three products resulting from this operation, gluten, starch liquor, and wash waters, can be further purified by conventional procedures. Methods of purification will depend on the end use of the particular product. For example, the gluten can be dried by various methods to produce either a denatured or undenatured product. The starch milk could be fermented without further treatment, or it could be tabled or centrifuged to yield a prime starch product. The insoluble material present in the wash water can be removed by filtration or centrifugation, and the clarified liquid used in the batter makeup or cutting stages to keep the water requirement of the process to a minimum. The solids, being primarily starch, can be combined with the starch fraction.

TABLE I

BATTER PROCESS RUNS — FLOUR COMPOSITION, OPERATING DATA, PRODUCTS ANALYSES, AND RECOVERIES

	Flour Type					
	Patent, Soft White Winter	Patent, Soft Red Winter	Patent, Hard Red Winter	Patent, Hard Red Spring	First Clear, Hard Red Winter	Second Clear, Hard Red Winter
Flour composition:						
Protein, %, MFB[a]	9.2	11.2	14.3	14.6	16.6	17.9
Ash, %, MFB	0.39	0.34	0.41	0.41	0.79	1.13
Solubles, %, MFB	5.1	4.9	6.3	6.3	7.4	8.9
Operating data:						
Flour feed, lb., MFB	96.2	88.6	87.2	87.2	86.7	84.6
Total mixing water, lb/lb dry flour	0.86	0.90	1.11	1.11	1.17	1.55
Cutting water, lb/lb dry flour	1.56	1.41	1.44	1.44	1.44	1.48
First screen, mesh	150[b]	150	150	150	150	60[c]
Second screen, mesh	100[d]	100	100	100	100	60
Products:						
Gluten fraction, lb.	25.0	35.0	44.5	50.0	48.0	52.5
Solids, %	33.4	36.4	35.1	35.2	37.6	31.5
Protein, %, MFB	81.0	81.0	80.7	79.6	83.7	76.4
Starch milk, gal.	76.5	60.0	56.5	54.9	56.5	68.6
Solids, lb/gal	0.95	1.01	0.97	0.98	0.98	0.86
Protein, lb/gal	0.021	0.019	0.022	0.024	0.028	0.038
Wash water, gal.	130	150	138	118	148	175
Solids, lb/gal	0.098	0.083	0.133	0.146	0.107	0.033
Protein, lb/gal	0.0066	0.0030	0.0054	0.0044	0.0053	0.0025
Recoveries:						
Solids recovery, %, MFB	97.0	94.1	98.8	97.2	98.7	95.3
Protein recovered in gluten fraction, % of total protein in flour	72.1	84.0	84.1	85.9	83.6	80.0

[a] MFB — Moisture-free basis.
[b] 0.0041-in. openings.
[c] 0.0092-in. openings.
[d] 0.0065-in. openings.

Materials and Methods

A number of commercial, untreated flours milled from various types of wheat were used in testing the continuous batter process pilot plant. Analyses of the flours used are found in Table I.

The moisture of each flour was determined by drying a sample for 4 hours at 110°C., under a vacuum of 28 in. of mercury. Protein ($N \times 5.7$) was determined by the Kjeldahl-Gunning-Arnold Method (1). Ash determination was carried out according to the official method of analysis of the A.O.A.C. (3). The solubles determination was made by shaking 1 g. of flour in 100 ml. of distilled water intermittently for 30 minutes, centrifuging, and then analyzing an aliquot of the supernatant for total solids.

Approximately 100 lb. of flour, as received, were processed in each of the tests, and each run was for a period of 1 hour. Operating variables and recovery and analyses of the products are also listed in Table I. The excess water in the starch milk over that added in the mixing and cutting stages is due to the water sprayed on the gluten separating on the first screen. The wash water is the water added to the gluten washing pump. The amount of wash water used in these 100-lb. tests is considerably more than is required when running the pilot plant at capacity.

Results and Discussion

For these tests all variables except the amount of mixing water were held as constant as possible. The amount of water necessary to give the desired batter characteristics varied widely with the particular flour being processed. The amount of mixing water required increased with the protein content of the flour.

Although there were minor variations in composition of the products recovered, they were quite similar considering the wide range of flours processed. Protein content of the gluten recovered from each of the flours was about 80%. This value indicates that the pilot plant could be used to process most flours and would produce glutens of fairly high purity. The protein content of the starch milk was less than 5%, dry-solids basis, and in most cases below 3%. Its composition is such that it may be fermented directly, centrifuged, or tabled to yield a prime starch product.

Recovery of solids and protein in the gluten fraction is based on recovery of protein in the starch and wash waters. This calculation was found to be more reliable than using wet gluten as a base. Gluten in a wet state is very difficult to sample and analyze satisfactorily, and

the gluten recovery based on its analysis was usually unreasonably high. The recovery of total protein in the gluten fraction was 80%, or higher, with all the flours processed except that milled from soft white winter wheat. This flour was quite low in protein and its gluten was soft and difficult to collect on the screens. With further experimentation, it is believed that recovery of protein in this gluten can be increased. The recovery of dry solids was good, particularly considering that tests were of only 1 hour duration. A higher recovery would be expected from full-scale plant operations.

Wet gluten from the process was dried carefully under vacuum at 95°F. (35°C.) and subjected to baking tests; results indicated that the gluten was not heat-damaged during processing and could be used in commercial drying processes to prepare dried vital gluten of excellent quality. Similarly, starch from the process was subjected to microscopic examination which showed no evidence of heat-damage. The soluble fraction from the process was recovered by means of low-temperature evaporation and spray drying; it yielded a product containing about 90% water-soluble solids and having a protein content as high as 26%, with water solubility of the protein ranging from 68 to 79%. These dried solubles should have potential use as nutrients in foods, feeds, and fermentation media, or as a source of albumins and globulins.

Operation of the continuous process has shown it to be easily controlled and amenable to instrumentation, so that it can be carried out with a minimum of requirement for labor, power, and water. Gluten curds can be kept small, taken directly to be dried, or can be dispersed in liquid, depending upon the drying process selected or the end use of the gluten. The process yields products of uniform prime quality and composition. Because of the continuous nature of the process, holdup of materials being processed is kept at a minimum, and enzymatic or microbiological degradation of fractions can be minimized by proper design and streamlining of the process components. Another advantage of the continuous batter process is the possibility of obtaining the solubles fraction in a more concentrated form than is usually possible with processes now in use commercially. Utilization of the batter process should permit the economical recovery of this solubles fraction, which is usually discarded even though it amounts to as much as 10% of the total flour weight.

Effects of other process variables on recovery of flour protein in the gluten fraction are being investigated and will be reported, together with cost estimates on the process, in future publications.

Acknowledgment

The authors express their appreciation to J. G. Pemberton and L. H. Burbridge for their assistance in constructing and operating the pilot plant. The assistance of G. T. Van Duzee in carrying out the many analytical determinations is gratefully acknowledged.

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VITAL WHEAT GLUTEN BY DRUM-DRYING
I. Effect of Processing Variables[1]

V. F. Pfeifer, Charles Vojnovich, and R. A. Anderson

ABSTRACT

Dry vital wheat gluten was prepared by atmospheric drum-drying of wet gluten dispersed in dilute acid. The wet gluten is dispersed in acid, preferably acetic, at a pH in the range of 4.5 to 5.1 and at a solids content in the range of 10 to 25%. Steam pressure in the drums is relatively unimportant, but complete dispersion of the gluten is essential in order to produce a vital material. Products of good quality were obtained over a wide range of conditions, as shown by baking, mixing, solubility, dough expansion, and rehydration tests. Gluten of good quality was produced when an acetic acid dispersion at a pH of 4.7 and containing 16.5% solids, prepared in a Waring Blendor, was drum-dried at atmospheric pressure with steam in the drums at 40 p.s.i.g. The bulk density of the ground material was about two-thirds that of vacuum-dried gluten. Approximate cost calculations indicate a plant production cost between 3 and 4 cents per lb. to produce 6,000,000 lb. of dried gluten per year.

Vital wheat gluten is becoming increasingly useful as an ingredient in food preparations, especially for fortifying flours of low protein content or quality. Vital wheat gluten is being used in diabetic preparations, in geriatric, dietetic, and baby foods, and in cereals. It also has potential use as a raw material for producing industrial chemicals.

Separation of wheat gluten from flour is usually accomplished on a commercial scale by one of two processes. In the Martin, or dough-ball process (3), wheat flour is kneaded to a stiff dough and the starch is washed out of the dough with water. In the batter process as described by Dimler (4), Hilbert *et al.* (5), and Rist (11), a smooth batter is prepared from wheat flour and water, and this mixture is washed with water so that the gluten is left behind in the form of curds that may be easily screened and washed.

Production of dried vital gluten on an industrial scale presents many difficulties because of the sticky nature of wet gluten after separation from flour. Commercially, wet gluten is dried in vacuum ovens at relatively low temperature after it has been cut into pieces or otherwise subdivided; wet gluten is mixed or beaten with dried gluten from the process, and the mixture is air-dried in a flash dryer employing a stream of warm water-absorbing air (10); or a dispersion of wet gluten containing about 10% of dry gluten is spray-dried as described by McConnell (8). Other potential processes for producing dry vital gluten

[1] Manuscript received February 17, 1958. Contribution from the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois. Presented at the 43rd annual meeting, Cincinnati, Ohio, April 1958.

on a commercial scale include tunnel-drying a thin ribbon of wet gluten extruded on a belt as described by Slotter and Delap (13), and drum-drying from ethanol dispersion as described by Tuomy and Slotter (14). These processes yield dried gluten retaining many properties of the original wet gluten. However, each method possesses some disadvantages in requiring elaborate equipment, excessive labor or power, or in being impractical for a high rate of production.

Pence *et al.* (9) recently investigated the rate of denaturation of wheat gluten as related to moisture content, pH, and salt concentration. Their investigations show that the rate of denaturation is reduced considerably when the pH of the mixture to be dried is below 6.0 and that very little denaturation is encountered at a moisture content below about 10%. From these findings we concluded that a suitable method for drying wet gluten should minimize the heating time and be carried out at a pH below 6.0. These conditions can be attained by dispersing wet gluten in dilute acid and by drying it at atmospheric pressure on a conventional steam-heated drum dryer.

This paper describes drum-drying of wet gluten from dispersion in very dilute acid, using a small pilot-plant drum dryer. Variables investigated were dispersion method, pH, type of acid used, drying temperature, solids content, and type of flour from which the gluten was obtained.

Equipment and Methods

Equipment. Drum-drying experiments, all at atmospheric pressure, were carried out on a Buffalo Machine and Foundry Company Laboratory Double Drum Dryer,[2] with rolls 6 in. in diameter and 7⅝ in. long constructed of cast iron, chromium plated. The total area of the drums was 2.0 sq. ft. Steam pressure was adjustable in the range of 2 to 120 p.s.i.g., rotational speed of the drums was variable between 2 and 14 r.p.m., and clearance between the drums was variable.

Liquid dispersions of wet gluten in dilute acid were made in a 1-gal. Waring Blendor Model CB-3. To make a dispersion, the acid solution was placed in the bowl and the gluten was added in the form of small pieces about ½ to 1 in. in diameter. Total dispersing time was about 4 minutes. After dispersion, the liquid was cooled below 122°F. (50°C.).

High concentrations of gluten in dilute acid were prepared in a stainless-steel laboratory mixer or kneader equipped with a pair of sigma blades, one of which rotated at 60 r.p.m. and the other, at 40

[2] Mention of proprietary or trade names is not to be construed as an endorsement over other products of a similar nature, but is made for information purposes only.

r.p.m. The capacity of the mixing bowl was about 1,200 g. Mixing was usually carried out for 60 to 120 minutes.

Homogenization of the kneaded mixture before drum-drying was made in some experiments. This was done by passing the mixture through a 3-in. Premier colloid mill operating at 17,000 r.p.m.

Dried gluten was ground in a standard Mikro-Samplmill equipped with a screen having 0.020-in. holes. To prevent overheating of the gluten the mill was jacketed with solid carbon dioxide, or finely ground solid carbon dioxide was fed along with the gluten so that the ground product was always below 122°F. (50°C.).

Experimental Techniques. Glutens were separated from various types of flour by doughing and hand washing, or by applying the batter process to the flour to be fractionated. All of the wet glutens tested contained 65 to 70% moisture, with 80 to 85% protein on a dry basis, and were in the pH range of 6.5–7.0. Each gluten was dispersed in acid by means of the blender, kneader, or combination of kneader and homogenizer; the dispersion was drum-dried under the selected conditions, and the resulting product was ground in the Mikro-Samplmill at a temperature below 122°F. (50°C.). Experiments were carried out to show how variations in the method of dispersion, pH of the dispersion to be dried, type of acid used in making the dispersion, drying temperature, solids content of the dispersion, and type of flour from which the gluten was obtained affected the drying operation and the quality of the dried product.

In each experiment, some of the wet gluten was also dried at 95°F. (35°C.) at 1 cm. mercury absolute pressure for 16 hours, and the dry material was ground below 122°F. (50°C.) to yield substantially undenatured gluten for comparison with the glutens produced by drum-drying. Each set of experiments thus contains a vacuum-dried preparation serving as a control for that group, since the wet glutens varied somewhat even when separated from the same flour. In three experiments wet gluten was drum-dried at atmospheric pressure without acidification or dispersion to yield denatured gluten for comparison.

Analytical Methods. Moisture in the dry materials was determined by heating 2-g. samples at 100°–110°C. for 4 hours in a vacuum oven operating at 29 in. of mercury.

Protein ($N \times 5.7$) was determined by the improved Kjeldahl method for nitrate-free samples (1).

The quality of the dried gluten was estimated from the nitrogen solubility in 0.1*N* acetic acid, the expansion of fermented dough prepared from wheat starch and gluten, the measurement of the power required in mixing a blend of the gluten and soft wheat flour, and the

stretch and feel of the gluten when rehydrated with water or buffer solution. In addition, baking tests were carried out on the various glutens using the bread-baking procedure described by Larmour *et al.* (7), with flour made up of 88 parts of soft winter wheat flour and 12 parts of the gluten under test.

In determining the nitrogen solubility of the dried gluten, 1 g. was transferred to a 100-ml. volumetric flask, 25 ml. of 0.1*N* acetic acid were added, and the mixture was shaken thoroughly and allowed to stand for 18 hours. The mixture was diluted to 100 ml. with 0.1*N* acetic acid, centrifuged, and nitrogen determined in an aliquot of the supernatant. Results are expressed as the percentage of the total nitrogen soluble in 0.1*N* acetic acid.

A dough expansion test was carried out by mixing 15 g. of gluten and 80 g. of wheat starch, and adding the mixture to about 60 ml. of water containing 5.0 g. of dextrose and 1.0 g. of viable dried yeast. The dough was mixed thoroughly, placed in a 16-oz. jar, allowed to rise at 86°F. (30°C.) for 3 hours, and the volume was measured. When testing a gluten that was drum-dried from acid dispersion, 15 ml. of the water were replaced with 15 ml. of 1% dipotassium phosphate buffer solution in order to maintain the pH above 5.5.

A mixing test was carried out in a 50-g. Brabender Farinograph mixer bowl fitted with a 1/6-h.p., 50-r.p.m., 110-v., 60-cycle AC gear-motor. The motor was wired in series with a 5-ohm resistor and operated under 70 to 80 volts so that the power factor approached 1.0 and the power was essentially proportional to the current. The AC voltage drop across the 5-ohm resistor, which is proportional to the current through the mixer motor, was measured by converting it to a DC voltage, amplified, and the output recorded on a 0–1 ma. recording milliammeter. This method is essentially the one used by Anderson and Lancaster (2) to record the mixing characteristics of batters made from wheat flour and water. After 20 g. of gluten and 15 g. of soft wheat flour were mixed in the bowl and the instrument zeroed, 25 ml. of water were added (1% dipotassium phosphate solution in the case of gluten dried from acid dispersion), and the power requirement of the mixer was recorded for 8–10 minutes. The curve obtained in the mixing test shows the rate of strengthening of the gluten-flour mixture, which is related to the rate of hydration of the gluten. Damaged glutens develop strength very slowly and reach low final values, whereas undenatured glutens develop maximum strength in from 3 to 5 minutes.

In a rehydration test, 5 g. of ground gluten and 10 ml. of water (1% dipotassium phosphate solution in the case of gluten dried from acid

dispersion) were mixed thoroughly, and the stretch and feel of the resulting elastic mass were compared with those of vacuum-dried gluten prepared from the same batch of wet gluten. Results are expressed on an arbitrary scale of 0, 1, 2, 3, or 4. Zero is used to describe materials so completely denatured that the gluten will not agglomerate, and 4 describes materials that behave like the vacuum-dried product originating from the same wet gluten.

Results

Effect of Dispersion Methods. Experiments were made on three different methods of dispersing wet gluten in dilute acetic acid: dispersion by means of the Waring Blendor, dispersion by means of the

TABLE I

EFFECT OF DISPERSION METHOD ON DRUM-DRYING GLUTEN

Dispersion Method	Feed			Dried Gluten				
	Solids Content	pH	Drying Temperature	Rehydration Test	N_2 soluble in 0.1*N* HAc	Dough Expansion, Percent of Vacuum-Dried Control	Mixing Test	Loaf Volume, Percent of Vacuum-Dried Control
	%/wt		*°F*	*arb. units*	*%*		*arb. units*	
	Gluten from clear grade flour; ash content 0.95%, protein content 14.0% (d.b.)							
Control [a]				4	95	100	62	100
Waring Blendor	18.0	4.7	267 [c]	3	95	118	48	110
Mixer and homogenizer	22.0	4.6		2	86	112	46	96
Mixer (no puddle)	22.0	4.8		1	78	96	44	76
Mixer (puddle)	23.2	4.6		1	71	74	53	66
Drum-dried as is [b]	33.0	7.2		0	54	45	21	50
	Gluten from untreated patent flour; ash content 0.41%, protein content 14.3% (d.b.)							
Control [a]				4	96	100	54	100
Waring Blendor	16.5	5.0	287 [d]	3	83	103	53	94
Mixer and homogenizer	20.0	5.0		2	75	86	46	90
Mixer	20.0	5.0		1	74	86	49	78
Drum-dried as is [b]	33.0	6.9		0	54	40	17	58
Control [a]				4	95	..	..	100
Waring Blendor	16.5	4.6	287 [d]	4	97	..	..	105
Mixer and homogenizer	20.0	4.6		3	85	..	..	98

[a] Vacuum-dried at 95°F. (35°C.) without acidification or dispersion.
[b] Drum-dried without acidification or dispersion.
[c] 131°C.
[d] 142°C.

laboratory sigma-blade mixer, and dispersion by homogenization in the colloid mill of a kneaded mixture. Results of the experiments are shown in Table I. Analyses of vacuum-dried glutens and denatured glutens produced by drum-drying without acidification and dispersion are included for comparison. Drum-dried glutens contained between 4.5 and 7.2% moisture. Results of the experiments show that the method of dispersion is extremely important.

When the gluten was thoroughly dispersed, as it is with the Waring Blendor, the resulting dispersion could be drum-dried with little loss of baking strength, acetic acid solubility, and dough expansion properties. However, some hydrolysis evidently occurred as evidenced by the reduced mixing strength. When the acid was crudely mixed with the wet gluten in the laboratory kneading-type mixer, the resulting dispersion was in the form of a soft paste which could be drum-dried at an increased rate, but the resulting dried gluten was low in quality. However, this dispersion could be passed through a colloid mill for homogenization, and the resulting dispersion drum-dried to yield gluten of good quality, although not quite as good as that produced with the Waring Blendor. When the dispersion from the laboratory kneading-type mixer was dried in a manner such that the puddle space between the rolls remained empty, the dried product was somewhat higher in quality, but it still was not quite satisfactory for baking purposes.

Effect of Dispersion pH. Results of experiments in which the pH of the dispersion was varied over a limited range by means of varying the quantity of added acetic acid are given in Table II. All the dispersions were made in the Waring Blendor. The effect of gluten concentration in the feed can be observed in the second and third groups of tests in which the feed contained 20% and 16.5% solids, respectively. The drying temperature used in the tests was 267°F. (130°C.). Drum-dried glutens contained between 3.8 and 8.4% moisture. Vacuum-dried controls and one denatured gluten produced by drum-drying without acidification and dispersion are included for comparison.

The upper limit of the pH range, 5.1, was chosen because it was difficult to disperse the gluten in acetic acid in the concentration range of 15 to 20% solids if the final pH was much above this value. Experiments indicated that the optimum pH of the dispersion for drum-drying was dependent upon the type of flour from which the gluten was obtained and upon the concentration of the gluten in the dispersion. With a lower gluten concentration in the dispersion, a higher pH could be tolerated to yield the same quality of gluten. Results indicate that most glutens prepared from clear and patent flours can be drum-

TABLE II

EFFECT OF pH OF WARING BLENDOR DISPERSION ON DRUM-DRYING OF GLUTEN

Feed		Dried Gluten				
pH	Solids Content	Rehydration Test	N_2 soluble in 0.1*N* HAc	Dough Expansion, Percent of Vacuum-Dried	Mixing Test	Loaf Volume, Percent of Vacuum-Dried
	%/*wt*	*arb. units*	%		*arb. units*	
	Gluten from clear grade flour; ash content 0.95%, protein content 14.0% (d.b.)					
Control [a]		4	95	100	62	100
5.1	16.1	4	92	118	49	107
4.9	16.1	3	95	116	48	
4.7	16.1	3	94	113	47	
4.5	16.1	3	96	108	46	107
	Gluten from untreated patent flour; ash content 0.41%, protein content 14.3% (d.b.)					
Control [a]		4	96	100	51	100
6.7 [b]	33.0	0	54	38	17	58
5.1	20.0	1	71	95	43	76
4.9	20.0	2	71	90	43	84
4.7	20.0	4	93	87	42	91
4.5	20.0	3	80	84	40	94
Control [a]		4	96	100	56	100
5.1	16.5	2	76	93	51	96
4.9	16.5	3	80	86	50	100
4.7	16.5	4	86	81	46	105
4.5	16.5	4	88	90	46	101

[a] Vacuum-dried at 95°F., no dispersion.
[b] Drum-dried without acidification or dispersion.

dried satisfactorily if dispersed in acetic acid in the pH range of 4.5 to 5.1, but that some optimum value exists for each particular gluten.

Effect of Type of Acid Used for Dispersion. Experimental results, summarized in Table III, show the effect of the use of acetic, hydrochloric, phosphoric, and lactic acids for dispersing the gluten preparatory to drum-drying. All dispersions were made in the Waring Blendor. Drum-dried glutens contained between 3.1 and 7.4% moisture.

The results indicate that any of the named acids can be used. Presumably, in order to obtain optimum results, the pH would have to be adjusted in accordance with the kind of flour from which the gluten was obtained and with the concentration of gluten in the dispersion. Acetic acid was used in most of the experiments because of its widespread acceptance in food products.

Effect of Drying Temperature. Table IV lists the results of experiments in which the steam pressure to the rolls was varied from 25 to 85 p.s.i.g. All dispersions were made with acetic acid in the Waring Blendor. Drum-dried glutens contained between 3.5 and 8.4% mois-

TABLE III

ACIDS USED IN WARING BLENDOR DISPERSIONS OF GLUTEN PRIOR TO DRUM-DRYING

Acid Used for Dispersion	Feed			Dried Gluten				
	pH	Solids Content	Drying Temperature	Rehydration Test	N_2 soluble in 0.1N HAc	Dough Expansion, Percent of Vacuum-Dried	Mixing Test	Loaf Volume, Percent of Vacuum-Dried
		%/wt	°F	arb. units	%		arb. units	
	Gluten from clear grade flour; ash content 0.95%, protein content 14.0% (d.b.)							
Control [a]				4	96	100	63	100
Acetic	4.6	16.7	287 [b]	4	97	94	48	95
Hydrochloric	4.9	11.1		3	91	103	53	100
Phosphoric	4.6	11.1		3	89	94	49	93
Lactic	4.8	11.1		3	89	99	52	96
	Gluten from untreated patent flour; ash content 0.41%, protein content 14.3% (d.b.)							
Control [a]				4	96	100	51	100
Acetic	4.5	20.0	267 [c]	3	80	84	40	94
Hydrochloric	4.5	19.4		1	76	83	38	83
Phosphoric	4.6	19.1		2	80	70	40	86
Lactic	4.7	19.6		1	74	83	42	86
Control [a]				4	95	..	..	100
Acetic	4.7	16.7	267 [c]	3	94			87
Hydrochloric	4.8	16.7		2	82	..	..	94
Phosphoric	4.7	16.7		3	93	..	..	94
Lactic	4.7	16.7		3	82	..	..	100

[a] Vacuum-dried at 95°F. (35°C.) without acidification or dispersion.
[b] 142°C.
[c] 130°C.

ture. Results indicate that the quality of the drum-dried gluten is practically independent of the steam pressure in this range. However, the capacity of the dryer seemed to be somewhat higher when operated in the range of 20 to 50 p.s.i.g. because a thicker film of material formed on the rolls.

Effect of Solids Content of the Dispersion. During the course of the experiments the solids content of the dispersions varied from 10 to 25% by weight. Dispersions were made in the Waring Blendor in concentrations up to 20% by weight: this seems to be near the upper limit of concentration that can be effected by this piece of equipment. Dispersions up to 25% by weight were prepared in the laboratory kneading-type mixer equipped with sigma blades. Dispersions containing 25% solids were soft pastes that could be pumped, but which were very slow-flowing. In addition, the acid was not properly dispersed when the laboratory kneading-type mixer was used, even with 3 hours of mixing, and the drum-dried product from this material was con-

siderably denatured.

When materials from the mixer contained 20% solids, they were semi-liquid and flowed slowly. These mixtures, after passage through the colloid mill for homogenization and complete mixing with acid, assumed a more liquid condition, and could be handled easily by gravity flow and pumping. In general, better-quality dried products were obtained when the dispersions contained from 10 to 16% gluten. However, high-quality dried gluten could be produced from dispersions containing 20% gluten if care was taken to ensure thorough mixing of the acid and the gluten before drum-drying.

Effect of Flour Type. Wet gluten was separated from 10 different types of flour, and dried products were prepared by dispersion in dilute acetic acid in the Waring Blendor and then drum-drying. The flours included first and second clear flours from hard red winter wheat, and patent flours from hard red winter and hard red spring wheat. Difficulty was experienced in dispersing the gluten separated by hand washing with hard water from the clear flours, presumably because of their high ash content. This phenomenon was previously referred to by Sallans (12) and Kempf (6). It was necessary to reduce the pH

TABLE IV

EFFECT OF TEMPERATURE ON DRUM-DRYING WARING BLENDOR DISPERSIONS OF GLUTEN

Drying Temperature	Steam Pressure in Drums	Feed		Dried Gluten				
		pH	Solids Content	Rehydration Test	N_2 soluble in 0.1N HAc	Dough Expansion, Percent of Vacuum-Dried	Mixing Test	Loaf Volume, Percent of Vacuum-Dried
°F (°C)	*psig*		*%/wt*	*arb. units*	%		*arb. units*	
		Gluten from clear grade flour; ash content 0.95%, protein content 14.0% (d.b.)						
Control [a]		7.0	33.0	4	95	100	62	100
267 (130)	25	5.1	16.1	4	92	118	49	106
320 (160)	75	5.1	16.1	4	88	115	53	102
		Gluten from untreated patent flour; ash content 0.41%, protein content 14.3% (d.b.)						
Control [a]		6.5	33.0	4	96	100	56	100
267 (130)	25	5.1	16.5	2	76	93	51	96
328 (164)	85	5.1	16.5	2	76	90	52	98
Control [a]		6.7	33.0	4	96	100	51	100
267 (130)	25	5.1	20.0	1	71	95	44	76
328 (164)	85	5.1	20.0	1	71	88	40	74
Control [a]		6.7	33.0	4	95	..	..	100
267 (130)	25	4.7	16.5	3	94	..	..	87
328 (164)	85	4.7	16.5	3	85	..	..	89

[a] Vacuum-dried at 95°F. (35°C.) without acidification or dispersion.

of the mixture to 4.5 before some of these glutens could be dispersed, but even then the dried products were of low quality. However, when the clear flours were processed by the batter process, in which soft (zeolite-treated) water was used, the separated glutens were readily dispersible in dilute acid. When hand washing was carried out using soft or distilled water, the separated glutens were readily dispersible.

Dryer Capacity. The maximum capacity of the dryer when used to dry a 16.5% dispersion of gluten in dilute acid was about 1.4 lb. of product per hour per sq. ft. of drum area. This capacity compares with a production rate of about 2.5 to 3 lb. per hour per sq. ft. obtained in drum-drying wet gluten containing 33% solids with no suspension in acid, and with capacities of 2 to 4 lb. per hour per sq. ft. obtained in drum-drying other materials considered suitable for this type of operation. Even at this relatively low dryer capacity, drum-drying can be considered a practical method for drying wet wheat gluten. If devitalized rather than vital gluten is to be produced, the same equipment can be used, but the production of dried gluten will be 2 to 3 times as great for the same dryer area, and the drying cost will be reduced.

Product Characteristics. Vital wheat gluten prepared by the drum-drying processes is lighter in color and weight than material produced by vacuum drying. The bulk density of the ground powder usually varied from 0.25 to 0.35 g. per cc., whereas vacuum-dried materials usually weigh from 0.5 to 0.6 g. per cc. Because the process yields a fine powder, the development of rancidity may be a problem, especially in gluten originating from clear flours. This possibility may necessitate the use of an antioxidant or an inert atmosphere if the dried material is to be stored for a long period of time. Dried gluten prepared by drum-drying from acid dispersion requires a buffer for proper rehydration, since the pH of a mixture with water is usually below 5.5. Consequently, if the process is used for fortifying flours, superior results may be obtained if some mild alkaline buffer is employed. Gluten drum-dried from a low pH dispersion is readily dispersible in water and should have unique applications in industrial and food uses.

Discussion

These experiments indicate that wet wheat gluten can be drum-dried to a vital product. It is first completely dispersed in dilute acid to form a suspension having a pH in the range of 4.5 to 5.1 and containing up to 20% solids; then it is fed to an atmospheric drum-dryer heated with steam in the range of 20 to 80 p.s.i.g., and the resulting

dry material is pulverized to a size suitable for subsequent use.

In drying wet gluten by this process most of the power is required in the initial dispersion; power requirements for grinding are reduced because of the light, friable film of dried gluten produced on the drum-dryer. A rough preliminary cost estimate for dispersing wet gluten, drum-drying the dispersion, and grinding the dried product indicates a plant production cost of 3 to 4 cents per lb. at a dryer capacity of 1 lb. of dried gluten per hour per sq. ft. of roll surface, based on a plant production of 1,000 lb. of dried gluten per hour (6,000,000 lb. per year). This cost does not include interest, profits, income tax, or selling and administration expenses.

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HYDRATION CHARACTERISTICS OF RICE AS INFLUENCED BY VARIETY AND DRYING METHOD[1]

JOSEPH T. HOGAN AND RALPH W. PLANCK[2]

ABSTRACT

Milled rice samples of ten different varieties, three processed rices, and Century Patna variety dried at different temperatures were treated with water for 10–40 minutes at 70°–98°C. Determinations were made of the water absorption of the treated grains, of contents of undissolved solids, and of dissolved materials taken into the treating water. High values in each of these properties were obtained with varieties which cooked to a sticky condition and are generally considered undesirable. Low values were obtained with the long-grain varieties preferred for cooking and kernel cohesiveness. These varieties have high amylose contents and a tendency to yield a firm, dry, and nonsticky cooked product. The differences in water absorption and in dissolved and undissolved separated solids are directly related to the kernel cohesiveness and other cooking characteristics and are readily determined by the methods outlined.

There have been several attempts to relate chemical and physical properties of the rice kernel and of rice starch with hydration and processing characteristics of commercial milled rice. Rao (8) reported a close relation between the texture of cooked rice and its swelling number (water absorption) when cooked under certain standard conditions. Rice having a high swelling number is preferred by consumers since such rice is softer when cooked. This simple method has been in use in India for over a decade and has been considered to give reproducible results for Indian rices. Measurements by Halick and Keneaster (5) of the swelling numbers of rice revealed that the swelling numbers of long-grain varieties differed from those of other types; however, he found no definite differences among the long-grain varieties. While no definite conclusions were drawn, it appeared that the swelling numbers cannot be used to predict the cooking characteristics of popular American varieties. Rao *et al.* (9) found a close association between the amylose content of rice and its swelling number, i.e., varieties possessing the desired characteristics were found to have higher amylose contents ranging from 12 to 17% and to possess high swelling numbers.

Batcher *et al.* (4) standardized procedures for cooking rice in large and small quantities and developed tentative methods for measuring the physical and chemical characteristics of eight varieties of rice. The tests indicated that long-grain rices absorbed more water and the cooked grain had greater volume than either medium- or short-grain

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[2] Present address: 2667 Barbera Way, Rancho Cordova, California.

types. There was less solid material in the residual cooking liquids from the long-grain rices – Century Patna, Rexoro, and Texas Patna – and from Zenith, a medium-grain type, than from other varieties tested. Starch in the residual liquids appeared to be independent of grain type, and the starch in the residual liquids from Century Patna and Zenith was much less than in the liquids from other varieties. In a later study Batcher *et al.* (3) applied these methods to the evaluation of the cooking quality of 26 varieties or selections of milled white rice. Variety and environment as well as grain type influenced cooking quality. Long-grain varieties of rice tended to absorb more water than the other grain types, although there were exceptions. Variations in starch and total solids in the residual cooking liquids were credited to varietal factors although the source (location) of the variety appeared to be an influencing factor. Significant correlations were found among the objective chemical measurements and palatability evaluations.

The action of dilute alkaline solutions, generally potassium hydroxide, on the disintegration of immersed rice kernels has been proposed (11) as another means of distinguishing one variety of rice from another. Baldwin (2) and Lowry[3] developed an eosin color test which gave results that were related to some extent with processing behavior and which indicated that differences in chemical composition were responsible for differences in processing quality. More recently, Halick and Keneaster (5) reported that a starch-iodine blue test is useful for the same purpose and that the intensity of the blue color developed by the addition of a dilute iodine solution to water in which ground rice has been treated is indicative, in most cases, of the processing quality of the sample. Since amylose is the starch constituent which is responsible for development of the blue color and since the test temperature is 77°C., this test may really be a measure of the rupture of starch grains.

The present investigation was undertaken to characterize various aspects of rice quality by further elucidating the changes which occur during the hydration processes. This study considers the effect of time and temperature of water-treatment on the water absorption of the grains and on the content and composition of the rice constituents which pass into the water.

Materials

The rice used in the first phase of this study was of the Century Patna 231 variety and was grown near Crowley, Louisiana, in 1954. It

[3] Lowry, J. R. The eosin test for varietal differences in rice. Private communication. Mar. 10, 1955. General Foods Corp., Hoboken, N.J.

had been seeded April 8 and harvested August 31. One portion was air-dried at an ambient temperature of approximately 28°C. (83°F.) for 72 hours; a second was dried with forced air at 71°C. (160°F.) in a model Louisiana State University type dryer for 90 minutes. The samples were milled in the pilot mill of the University of Arkansas at Stuttgart, Arkansas, and stored 12 to 18 months before testing in an air-conditioned room held at 16°C. (60°F.) and 50–60% relative humidity.

Samples for the study of the influence of variety consisted of a representative sample of each of ten varieties furnished by John V. Halick, Rice Pasture Experiment Station, Beaumont, Texas. The rice was certified seed stock grown on the station under controlled conditions and was considered typical of the varieties selected; all were planted in March, 1955, except Zenith which was planted in July; all were stored 8 to 10 months at 16°C. (60°F.) and 50–60% relative humidity prior to testing. All samples of raw rice were classified to include only full grain kernels (head rice). For purposes of comparison, two commercial samples of processed rices, Minute and Converted (parboiled), and one experimentally prepared vacuum-expanded product, Guardite, were included among the rices tested.

Methods

Water Absorption. Five grams of rice were soaked in 50 ml. distilled water at room temperature for 30 minutes in a stoppered test tube, 25 by 200 mm. Subsequently, the test tube (unstoppered) was immersed in a constant-temperature bath for a predetermined period of time at a set temperature; samples were heated for 10, 20, 30, or 40 minutes at 70°, 80°, 86°, 92°, or 98°C. After heat-treatment, the contents of the test tube were emptied into a small circular basket of 20-mesh screen and the grains washed carefully with distilled water until the combined cooking and washing water amounted to 90 ml. The cooked grains were spread on filter paper for 5 minutes to remove surface moisture, then quickly transferred to a weighing dish and weighed. Moisture content was determined by drying in a forced-air oven for 16 hours at 104°C. and reweighing. The water absorption values reported are equal to percentage moisture content of the cooked and rinsed grains. They are expressed on a dry-weight basis and represent the average of duplicate determinations.

$$\text{Water absorption } (\%) = \frac{\text{weight of moisture in cooked rice}}{\text{weight of dry material in cooked rice}} \times 100$$

Undissolved Solids and Dissolved Materials in the Treating Water. The treating water and washings in the volumetric flask were diluted

to 100 ml., transferred to a 200-ml. centrifuge bottle, and centrifuged for 10 minutes at 2000 r.p.m. The clear, supernatant liquor was decanted into one tared weighing dish and the centrifuged solids washed into another. Both were dried at 104°C. for 16 hours and reweighed to give weights of dry materials.

Total Starch, Sugars, and Protein. Starch was determined by the anthrone-sulfuric acid method of McCready *et al.* (7) and total sugars by a volumetric modification of the Shaffer-Hartman method (10). Results are reported as glucose on the basis of a standard curve. Nitrogen was determined by a micro-Kjeldahl procedure (1) and converted to percentage protein by multiplying by 5.95, a factor reported by Jones (6) as being applicable to rice protein.

Results

Water Absorption by Immersion. Absorption of water by the rice kernel was found to increase throughout the longest test period, 30–40 minutes. The rate of absorption was higher, the higher the temperature of the treating water. Quite striking was the approximate doubling of the rate and ultimate amount of water absorbed as the temperature was raised merely from 80° to 86°C. or higher (Fig. 1). This effect is attributed to the fact that the temperature at which rice starch starts to swell is in the range of 74°–80°C.; at 80°C. it swells slowly, and, to a limited extent, at 86°C. and higher, swelling proceeds more rapidly, reaching its maximum at the elevated temperatures. Swelling begins somewhat lower than 70°C. for the Caloro and Blue Rose varieties (see Fig. 3).

The Century Patna 231 samples dried at 71°C. absorbed water somewhat less readily at 80°C. than did the air-dried samples, suggesting that the surface of the grains might have been altered by the elevated temperatures to which they had been exposed during drying (see Fig. 1).

When different varieties of rice were tested for water uptake at 70°C., measurements on each variety gave values for water absorption which were generally characteristic for the grain type of the variety (Figs. 2 and 3). The short-grain rice, Caloro, absorbed the most water; the medium-grain varieties, Zenith and Blue Rose, absorbed somewhat less; the long-grain varieties, Texas Patna, Texas Patna 49, Sunbonnet, Bluebonnet, Improved Bluebonnet, and Rexoro, absorbed the least water. Century Patna 231, a long-grain variety, was not true to type since it gave values intermediate between those of the typical long-grain varieties and those of the medium-grain varieties.

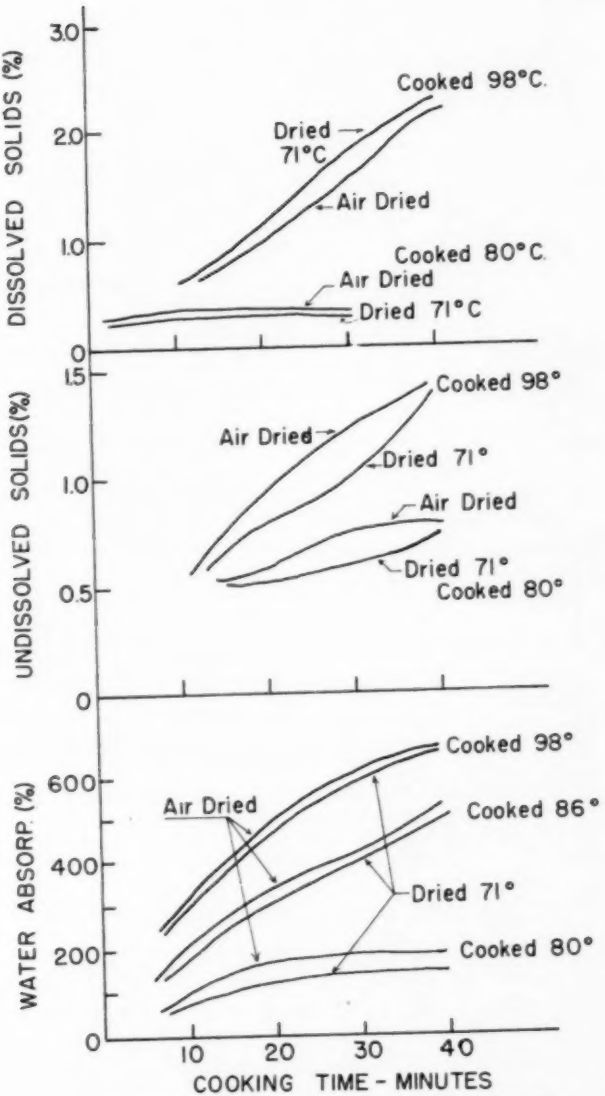

Fig. 1. Hydration characteristics of Century Patna 231 as influenced by drying temperature.

Of three processed rice products tested, Converted rice (parboiled) absorbed water to the same extent as the short-grain Caloro rice, and two precooked products, Minute Rice and Guardite, absorbed considerably more water. Starch is partially gelatinized in parboiled rice

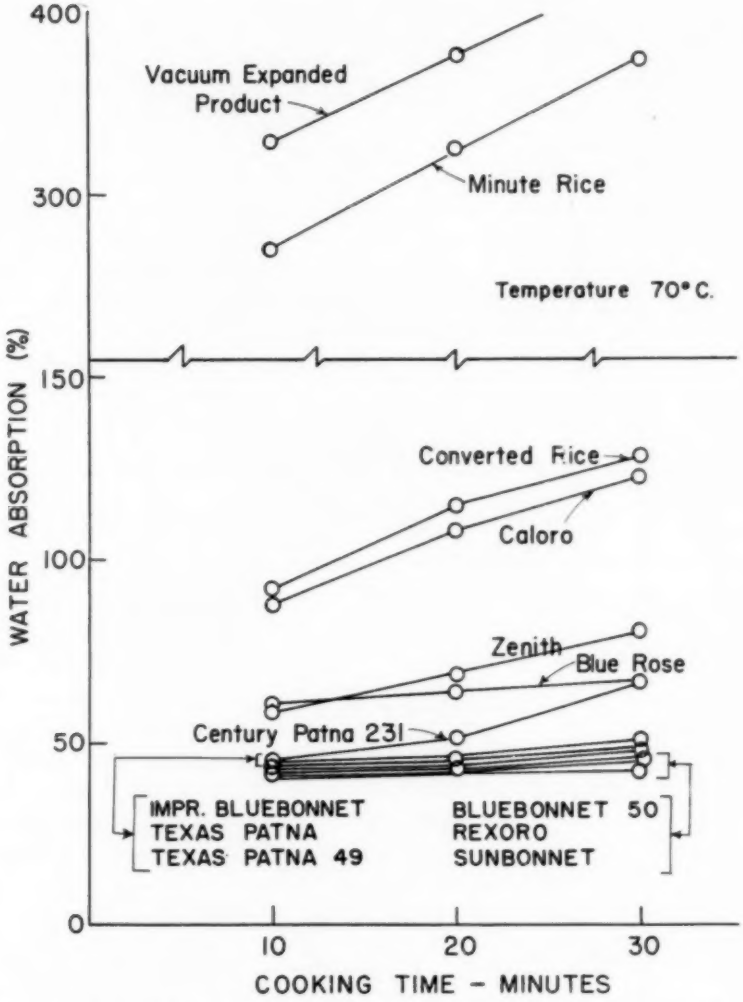

Fig. 2. Relationship of cooking time and water absorption of rices at 70°C.

and more completely gelatinized in Guardite (vacuum-expanded product) and Minute Rice.

Previously published values expressing water uptake have been based on the weights of the rice tested without regard to its initial moisture content or loss of solids during cooking. The results reported here as water absorption are the percentage of water in the cooked and rinsed rice expressed on the basis of dry weight of the undissolved and unseparated portion of the cooked kernel. Rices with low amylose con-

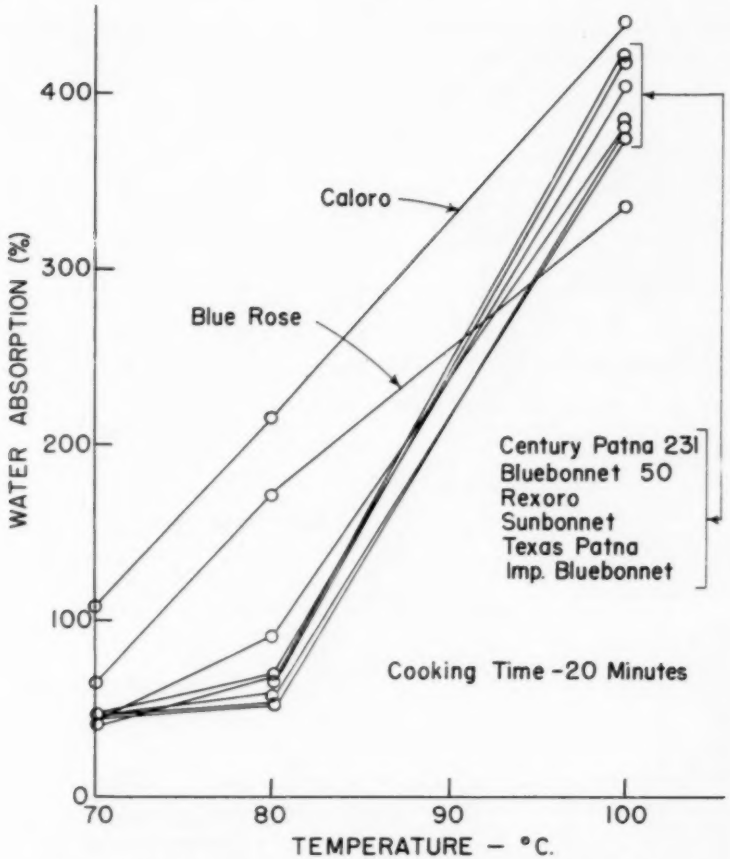

Fig. 3. Effect of cooking temperature on the water absorption of rices.

tents (13 to 15%) generally absorbed more water than did those with higher amylose contents (19 to 22%). This interpretation is based on amylose values determined potentiometrically by iodine titration at this laboratory (Table I) and elsewhere by Williams *et al.* (12).

Undissolved Solids in the Treating Water. The samples of Century Patna 231 rice dried at 71°C. gave up slightly less undissolved solids to the treating water than did comparable air-dried samples. This difference, like that observed in the water absorption, indicates that the surface of the grains was altered slightly by the higher drying temperature.

The trends revealed in Figs. 4 and 5 by loss of undissolved solids to the cooking water in the different varieties are again, in general, char-

TABLE I

VARIETAL DIFFERENCES IN AMYLOSE CONTENT AND WATER ABSORPTION OF RICE

VARIETY	GRAIN TYPE	AMYLOSE CONTENT	WATER ABSORPTION AT 70°C.		
			10 Minutes	20 Minutes	30 Minutes
		%			
Century Patna 231	long	13.6	46	52	67
Caloro	short (pearl)	13.8	88	108	123
Blue Rose	medium	14.7	60	65	67
Zenith[a]	medium	19.6	59	69	81
Bluebonnet 50	long	20.7	44	45	46
Improved Bluebonnet	long	22.5	40	44	51
Rexoro	long	22.8	42	43	46
Texas Patna	long	23.1	44	45	48
Sunbonnet	long	23.2	42	43	43
Texas Patna 49	long	23.4	42	46	46

[a] Seeding date in July; all other varieties seeded in March, 1955.

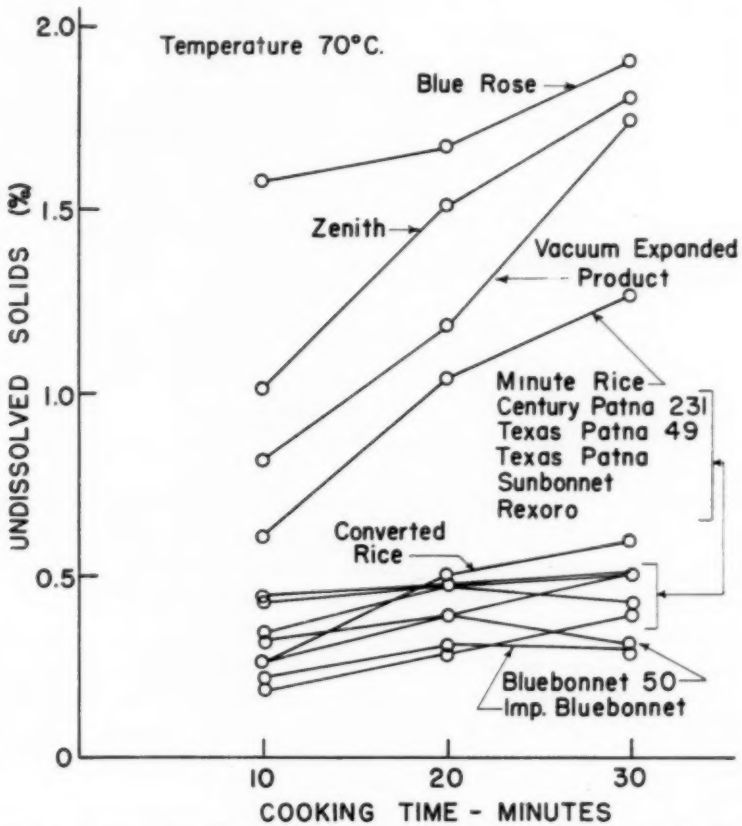

Fig. 4. Relationship of cooking time and quantity of undissolved solids lost to the cooking water at 70°C.

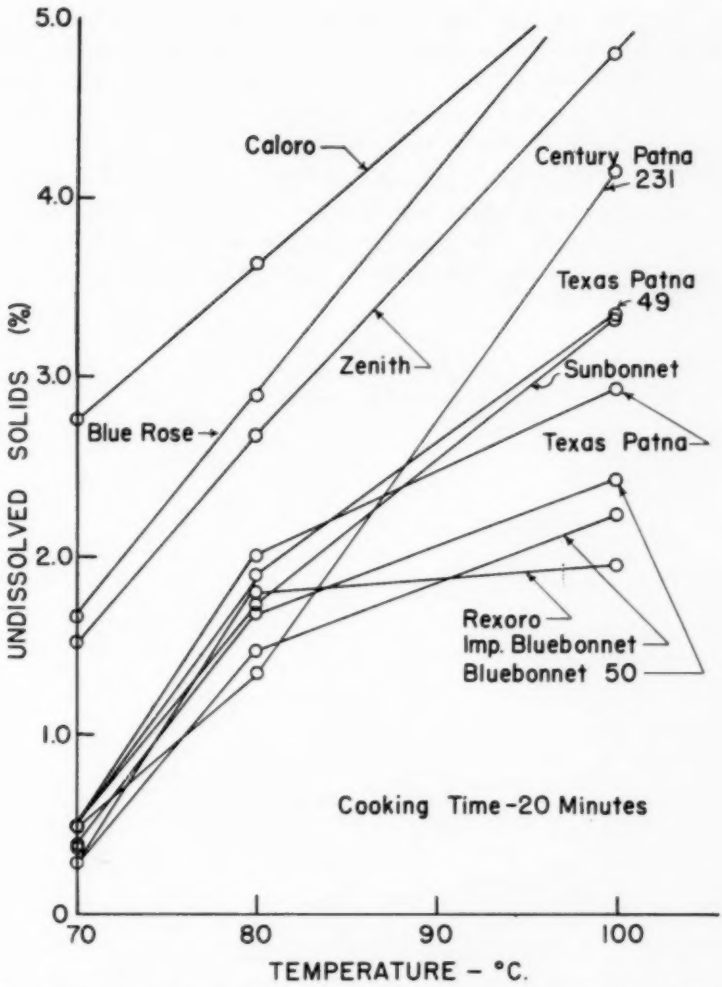

Fig. 5. Effect of cooking temperature on the quantity of undissolved solids lost to the cooking water.

acteristic of the grain types. The short-grain rice, Caloro, lost the most undissolved solids in excess of the amounts shown in Fig. 4; the medium-grain types, Zenith and Blue Rose, were next; the long-grain varieties showed the smallest losses to the treating water. Century Patna 231 differed from the other long-grain varieties, approaching the behavior of the medium-grain varieties, particularly when tested at 98°C. Losses of solids at 90°–100°C. parallel closely the relative order of the cooking properties of rices with respect to cohesiveness of the

grain. The Rexoro, Bluebonnet, Sunbonnet, and Texas Patna possess desirable cohesive characteristics. Century Patna 231 does not. The varieties whose loss (shown in Fig. 5) of undissolved solids exceeds that of Century Patna 231 at 98°C. cook to a consistency which is predominantly characterized by sticky or pasty grains.

Solids centrifuged from the treating waters obtained in tests on the two Century Patna samples, which were air-dried and dried at 71°C. (160°F.), were found to be composed largely of starch particles and to be similar in composition regardless of the drying history of the sample (Table II).

TABLE II

DISTRIBUTION OF STARCH, SUGARS, AND PROTEIN OF RICE COOKED AT 98°C. FOR 25 MINUTES

Distribution	Century Patna	
	Air-Dried	Dried at 71°C. (160°F.)
	%	%
Total starch		
Original rice[a]	89.3	89.7
Cooked grains[b]	86.8	87.2
Solubles in water[b]	1.36	1.35
Solids in water[b]	10.8	9.9
Total sugars		
Original rice[a]	0.41	0.43
Cooked rice[b]	27.4	26.2
Solubles in water[b]	63.1	64.7
Solids in water[b]	5.7	5.6
Protein		
Original rice[a]	8.85	9.50
Cooked grains[b]	96.5	96.7
Solubles in water[b]	1.19	1.02
Solids in water[b]	1.95	2.04
Solids centrifuged from water[a]	10.8	9.0
Dissolved material in water[a]	1.32	1.48

[a] Percentage based on the dry weight of the original rice.
[b] Percentage based on the amount of the component in the original rice.

Dissolved Materials in the Treating Water. Determination of dissolved materials in treating waters showed a marked increase as the test temperature was increased (Figs. 1 and 7). Values obtained after 30 minutes of treatment at 70°C. (Fig. 6) agreed reasonably well with known cooking characteristics; values obtained at 98°C. did not.

Treating waters obtained in one group of tests (Century Patna, 98°C., 25 minutes) contained the major part (63–65%) of the sugars originally present (0.41%) in the original rice. The water contained only small proportions of starch and protein. No effects attributable to drying history were observed.

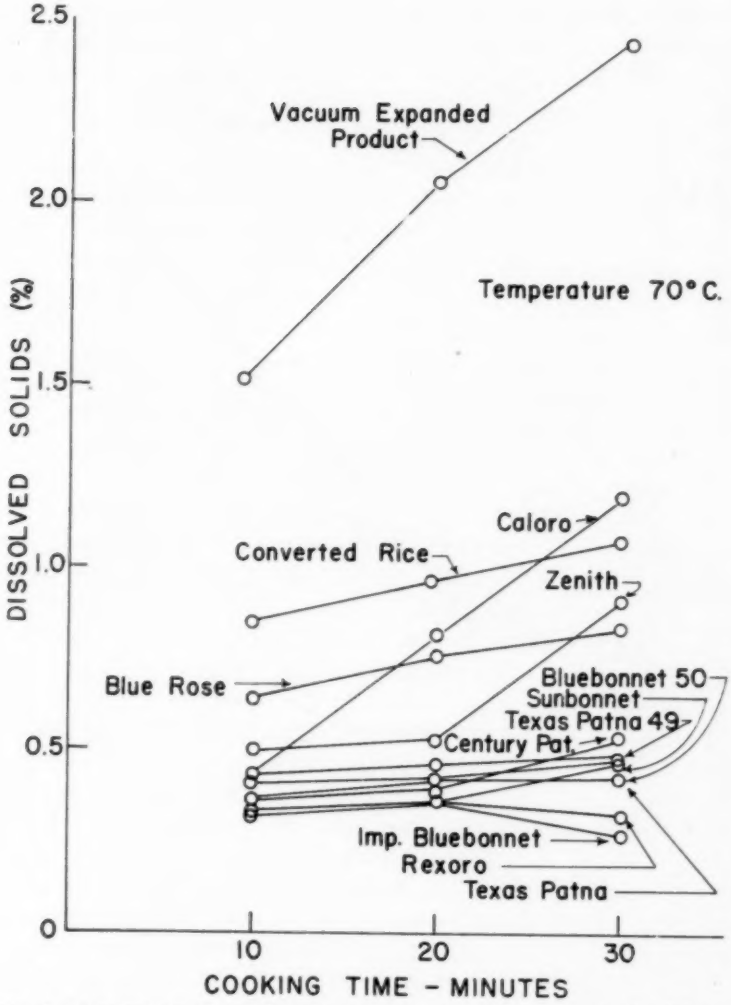

Fig. 6. Relationship of cooking time and the quantity of dissolved solids lost to the cooking water.

Discussion

The differences in the hydration characteristics of the three general types of rice, short-, medium-, and long-grain, agree in a general way with the cohesive properties and cooking quality of the grain, provided that the hydration is carried out at 70°C. for 20 to 30 minutes.

It was found, for example, that it is necessary to subject all the

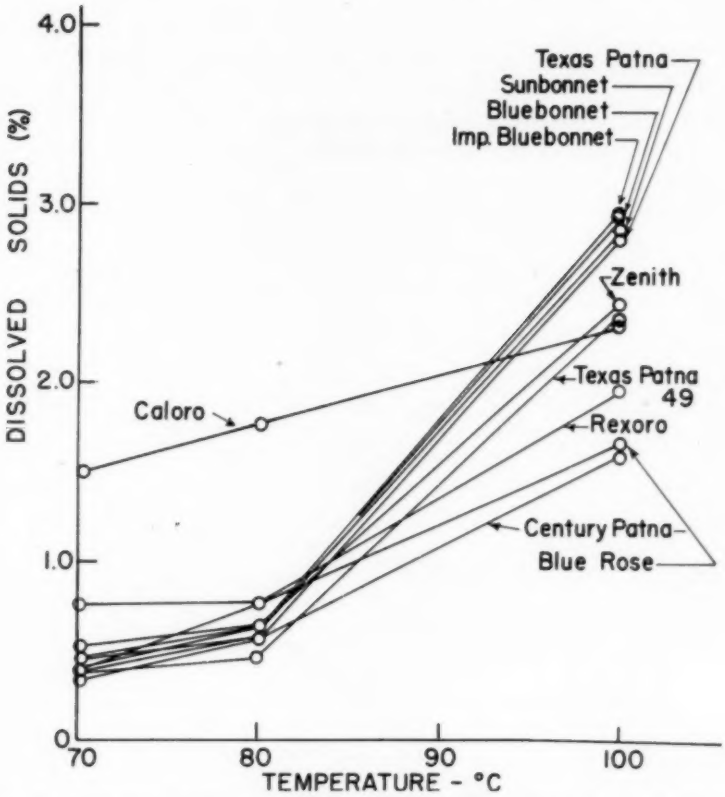

Fig. 7. Effect of temperature on the quantity of dissolved solids lost to the cooking water. Cooking time, 20 minutes.

grains to a presoak period at room temperature for 30 minutes, at which time all of them reached approximately the same moisture level of 36–38%. Preliminary work indicated that hydration differences found among rice samples, which had not been presoaked, were largely due to differences in moisture content of the interior of the grains resulting from incomplete wetting of the kernel.

The water absorption values of the hydrated kernels agreed with the cohesive properties and cooking characteristics and with the type of grain, provided that the tests were carried out at 70°C. for 20–30 minutes. High water-absorption values were indicative of poor cooking quality of the rices and stickiness when cooked; long-grain rices, which possess the superior quality of cooking to a dry, fluffy state without being sticky, were characterized by low water-absorption values. Quantities of undissolved solids in the treating water showed similar agree-

ment with cooking characteristics when the test conditions were at 98°C. for 30 minutes. This latter system showed better agreement of differences among various long-grain varieties than did the former.

Distinct differences in water-absorption values when determined at 70°C. for 20–30 minutes disappeared when the tests were made at temperatures above 70°C. It is thought that this masking of differences was from the effects of swelling and subsequent gelatinization of the starch at temperatures above 70°C. The amount of dissolved solids, while indicative, was not as consistent in differentiating processing and cooking characteristics.

By these tests, short-, medum-, and long-grain rices can be distinguished by physical determinations. Water uptake is greatest with the short-grain variety and least with the long-grain variety. Medium-grains fall at an intermediate level. The short-grains also have the greatest amount of undissolved solids; the long-grains, the least amount. Moreover, it is possible to distinguish readily between processed rices such as parboiled or quick-cooking rices and raw milled rices. The values for water uptake and for undissolved solids in these processed rices far exceed values for any of the raw milled rices, regardless of type.

Perhaps more important than the above-mentioned differentiation of grain types is the possibility of detecting long-grain varieties which are not true to type or deviate from the characteristics of other long-grain varieties. As an example, Century Patna behaves in its water absorption and in the amount of undissolved solids more like a medium-grain rice than the long-grain rice that it is. This might indicate that Century Patna differs, in cooking characteristics and in cohesiveness of the kernel, from the conventional long-grain rices. These tests might provide a quantitative measure of characteristics of rice with which to assign an order to the various rices in terms of properties that are closely related to cooking and processing characteristics. It is obvious that rice is a complex structure whose chemical and physical properties depend upon the many intrinsic properties of the constituents, which may be modified by environmental factors such as storage, conditioning, and curing. Differences in these intrinsic properties could have a profound influence on some of the characteristics that have been measured. It may be that a quantitative physico-chemical evaluation of rice would result in an ability to relate its performance with the chemical and physical properties of one or more of its constituents.

Acknowledgments

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staff for supplying the freshly harvested Rexoro rice and facilities for drying all of the samples. They wish to thank also L. C. Carter of the Arkansas Rice Growers' Cooperative Association, Stuttgart, Arkansas, and L. S. Ellis, Director of the Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, for making the pilot rice mill available for the milling of the samples used in this study.

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SOME EFFECTS OF THE MIXING PROCESS ON THE PHYSICAL PROPERTIES OF DOUGH[1]

C. J. Dempster and I. Hlynka

ABSTRACT

Results based on Extensogram® data for doughs mixed in nitrogen and in air showed that increasing mixing time produced a marked decrease in extensibility. Analysis of these data according to the structural relaxation procedure showed that mixing had very little effect on structural relaxation behavior of dough, but the effect of atmospheric oxygen occluded during mixing was pronounced. The effect of mixing *per se* appears to be distinct from and independent of the effect of oxygen. It is suggested that mixing decreases the size of the flow unit in dough.

The mixing of flour, water, and other ingredients to form dough is not a simple process. In addition to the mere physical blending, a number of other phenomena are recognized as associated with the mixing process. Baker and Mize (1,2,3) found that doughs could be mixed in inert gases or vacuum for prolonged periods of time without apparent deterioration. They described the role of gas occluded during mixing in relation to the origin of the gas cell in dough and to various stages of dough development in the mixograph. Freilich and Frey (8,9) established the beneficial effect of remixing on the properties of doughs showing excess bromate effect. They also investigated the role of oxygen in relation to mixing and dough development in bread doughs. Dempster, Hlynka, and Anderson (6) used the structural relaxation technique to assess the improving action of oxygen as judged by changes in physical properties of dough. Hawthorne and Todd (10) concluded that the improving effect of oxygen was a direct one on flour proteins. Cosgrove (4) related the uptake of oxygen from the air by flour batters with flour lipids, and Smith and Andrews (13) and Smith, Van Buren, and Andrews (14) in further studies associated the effect of oxygen specifically with its action through the oxidation of the polyunsaturated fatty acids by lipoxidase. Finally, Cunningham and Hlynka (5) showed that the amount of mixing received by the dough determined the rate of bromate decomposition in that dough.

The development of a mixer with a closed bowl for mixing dough in any desired atmosphere (11) offered the possibility of further examining the effect of the mixing action apart from other effects. An investigation was undertaken to examine the effect of the mixing process on the physical properties of dough as reflected by Extenso-

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gram® dimensions. The main effect of mixing was found to be on extensogram length rather than on extensogram height, which had provided the primary data for structural relaxation studies from this laboratory. The present paper summarizes the results.

Materials and Methods

The flour used in this study was an unbleached, improver-free, straight-grade sample commercially milled from a blend of Canadian hard red spring wheat. The protein content of the flour was 12.6%.

Although this study dealt mainly with extensogram length, the design of the experimental work was that usually employed in this laboratory for structural relaxation studies based on extensogram height (7). Moreover, some of the terminology originally developed in connection with structural relaxation studies has also been retained as aptly descriptive.

The experimental procedure was as follows. The flour samples were conditioned in nitrogen overnight before use to desorb and displace atmospheric oxygen originally present in the flour. Doughs (unleavened) were prepared from 200 g. of flour (14% moisture) and sufficient water and salt solution to give an absorption of 62.8% and a salt content of 1% (flour basis). The doughs were mixed in a GRL mixer with a closed bowl (11) for intervals of 1, 3, 5, 10, and 20 minutes. On the one hand, doughs were mixed in an atmosphere of nitrogen by first evacuating the mixer and then causing nitrogen from a commercial storage cylinder to flow through the mixing chamber during the entire period of mixing. For comparison, doughs were mixed in air by drawing air through the mixing chamber by means of a water aspirator at the rate of 1 liter per minute, the same as the rate of flow of nitrogen. Water at varying temperatures, depending upon the length of mixing, was circulated through the water-jacketed bowl so that doughs were taken from the mixer at $30 \pm 1°C$.

For each mixing time, three sets of experiments were made to give dough reaction times of 5 minutes, 2 and 4 hours. For each such experiment four separate mixes were required to give eight 150-g. test pieces of dough. At the end of a specified reaction time, the doughs were shaped on the Extensograph®, clamped into dough holders, and allowed rest periods from 5 to 120 minutes, at which time they were stretched. Extensibility measurements were the main primary data obtained from the extensograms. In addition, extensogram loads (heights) corresponding to a kymograph extension of 7 cm. (corrected) were recorded for analysis by the structural relaxation method.

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Results and Discussion

Effect of Mixing in Air and in Nitrogen on Dough Extensibility for Increasing Rest Periods. Figure 1 shows illustrative data for the change of extensibility with rest period for doughs mixed in nitrogen (left), and in air (right). The doughs were mixed for 1 minute and for 20 minutes; all doughs were given reaction times of 2 hours.

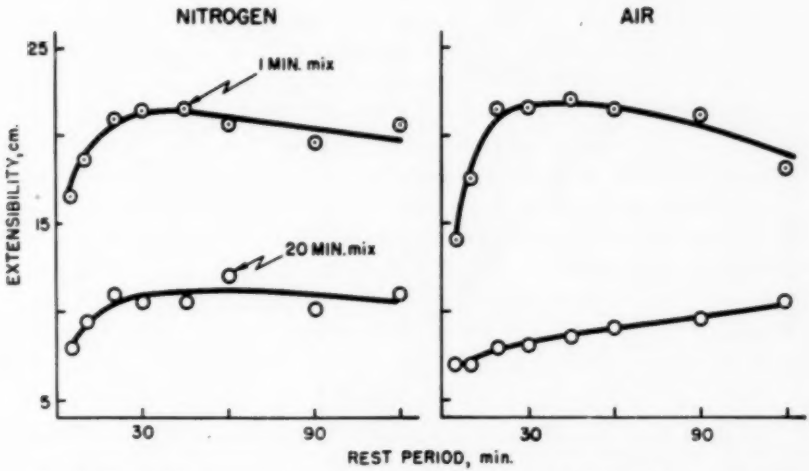

Fig. 1. Change in extensibility with rest period for doughs mixed for one minute and 20 minutes in an atmosphere of nitrogen (left) and in air (right). All doughs were given a reaction time of 2 hours.

The graphs show that the results for doughs mixed in nitrogen and in air are not as different as might have been expected. In general, the extensibility is small at short rest periods, increases at longer rest periods, and tends to level off as the properties of the relaxed dough stabilize. The curve for doughs mixed for 20 minutes in air appears somewhat anomalous, but it should be remembered that these doughs were extremely short from overoxidation with atmospheric oxygen. Their rate of relaxation would be expected to be very slow. The most striking difference is between doughs mixed for 1 minute and those mixed for 20 minutes; the extensibility was greatly decreased on prolonged mixing, whether the doughs were mixed in nitrogen or in air. Data obtained for other mixing times and other reaction times (not shown) confirmed these general effects of mixing on dough extensibility.

Effect of Mixing in Air and in Nitrogen on the Extensibility of Doughs Given Long Rest Periods. Average extensibilities at which re-

laxed doughs tended to stabilize were evaluated from curves such as those shown in Fig. 1 but for a wide range of mixing times and reaction times. While some approximation is involved in these average extensibilities, the data nevertheless show up the effect of mixing rather strikingly. Figure 2 shows the average final extensibilities plotted against mixing time. To focus the attention on the effect of short mixing time and to include long mixing, the time axis is shown on a logarithmic scale.

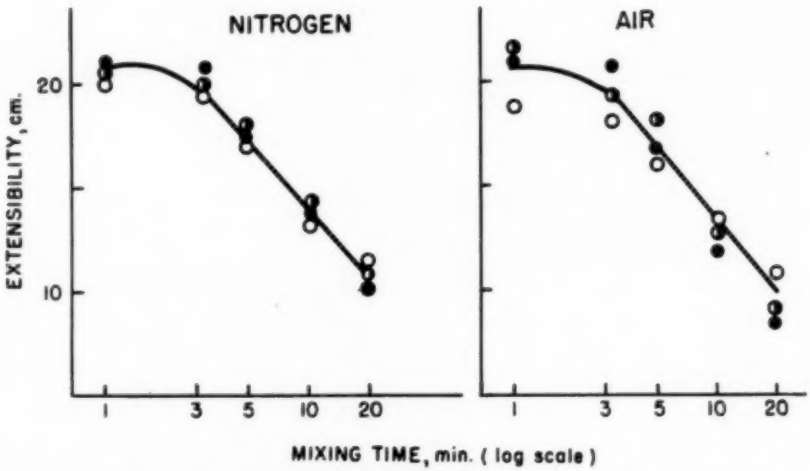

Fig. 2. Change in average extensibility of relaxed doughs with mixing time in an atmosphere of nitrogen (left) and in air (right). Data for 5-minute reaction time are shown as open circles, for 2 hours as half-filled circles, and for 4 hours as filled circles.

Data for three reaction times of 5 minutes (open circles), 2 hours (half-filled), and 4 hours (solid circles) are shown for doughs mixed in nitrogen and in air. The mean curves for mixing in nitrogen and air are essentially superposable, and both show that the effect of longer mixing in decreasing the extensibility of relaxed doughs is very marked indeed. Of the two factors, mixing and atmospheric oxygen, mixing decreased the extensibility of dough; oxygen had essentially no effect. This contrasts, as will be seen in the next paragraph, with the effect of these two factors on extensogram height and, hence, structural relaxation. It was oxygen that had a marked effect on extensogram height; the effect of mixing alone (in nitrogen) had essentially no effect on height.

Effect of Mixing on Structural Relaxation Characteristics of Doughs. Additional information on the effect of mixing action was obtained

from the measurement of extensogram height (load at constant sample extension) analyzed according to the structural relaxation procedure which has been fully described elsewhere (7). It was found that when doughs are mixed in nitrogen, increasing the mixing time had a negligible effect on structural relaxation. Accordingly, detailed results are not presented. Mixing the dough in air, however, was strongly reflected by changes in the relaxation constant and the asymptotic load, but since these data were essentially the same as those already published from this laboratory (6), they also are not given.

The two types of results taken together, those based on extensibility and those based on extensogram height (structural relaxation), are complementary. On the one hand, extensibility data showed clearly the effect of mixing alone (i.e., in nitrogen) but did not reflect the effect of oxygen occluded in the dough mixed in air. On the other hand, the structural relaxation method was not influenced by the amount of mixing (in nitrogen) that the dough received, but the effect of atmospheric oxygen occluded during mixing was strongly reflected in the structural relaxation constants of dough. It thus appears that the mixing process can decrease the extensibility of dough without influencing extensogram height (i.e., load at constant sample extension). This is in marked contrast to the generally observed inverse relationship between the two extensogram dimensions as is, for instance, obtained with bromate treatment; the greater the length or extensibility, the smaller is the extensogram height, and conversely.

Conclusions

Some suggestions can be made about the nature of the process which results in decreased extensibility in dough on prolonged mixing. Moore and Cravath (12), in their study on the mechanical breakdown of soap-base greases, showed convincingly by means of electron micrographs that the length of fibers was decreased with prolonged shear. On the basis of this analogy, it may then be suggested that the flow unit in dough is decreased during prolonged mixing. In other words, the effect of mixing on extensibility may be considered to be a physical effect, and the action of oxygen occluded during mixing may be reasonably assumed to be chemical. It may also be assumed that the consequence of such chemical action on dough would be reflected in changes in structural relaxation constants. Such a hypothesis is entirely consistent with the main result obtained in this study that the (physical) action of mixing on dough is distinct from and independent of the (chemical) effect of atmospheric oxygen occluded in the dough during mixing.

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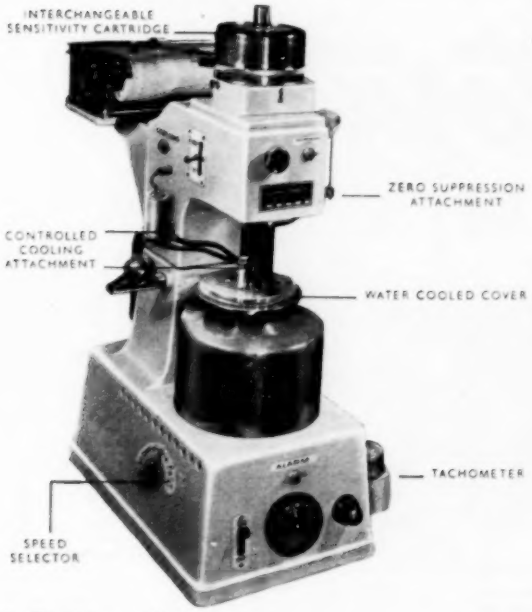

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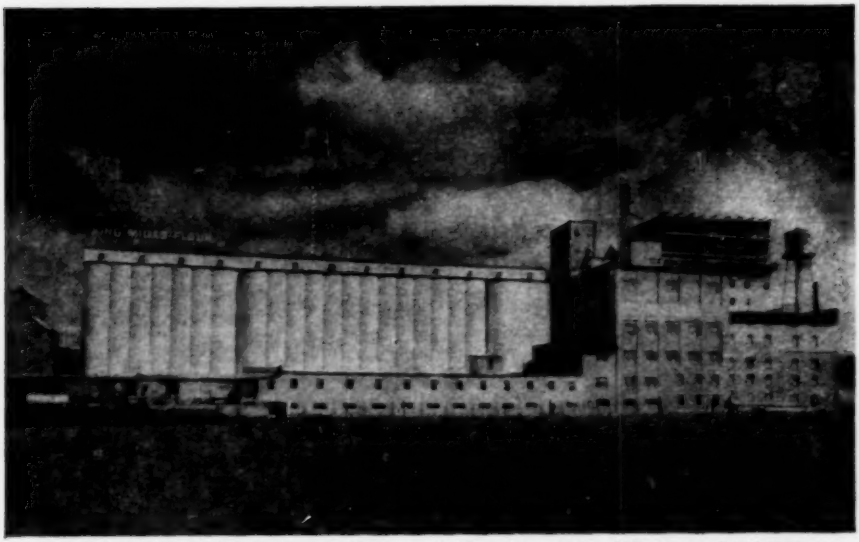

King Midas Flour Mills — Hastings, Minnesota. 9,400 sacks capacity. Mill storage 1,300,000 bu.

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